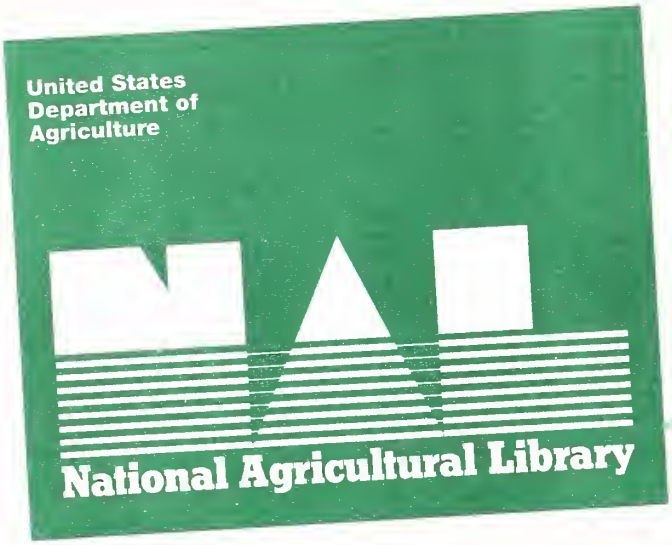
62nd Annual Western Veterinary Conference

Biotechnology and Applications in Veterinary Practice

United States Department of Agriculture

Animal and Plant Health Inspection Service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January 1990

Overview

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It is a tough job for a busy practitioner to keep up with new medical research, and there is no question that biotechnology findings will increase this burden. Since there seems to be no escape, the purpose of this symposium is to bring the practitioner up to date in this rapidly moving field. Gene splicing is the most powerful discovery since the splitting of the atom and rivals the development of the computer. The public and scientific press predicts a multibillion dollar industry. All of us will attempt to make the lectures as practical as possible and to relate biotechnology to your practice.

What Is DNA All About?

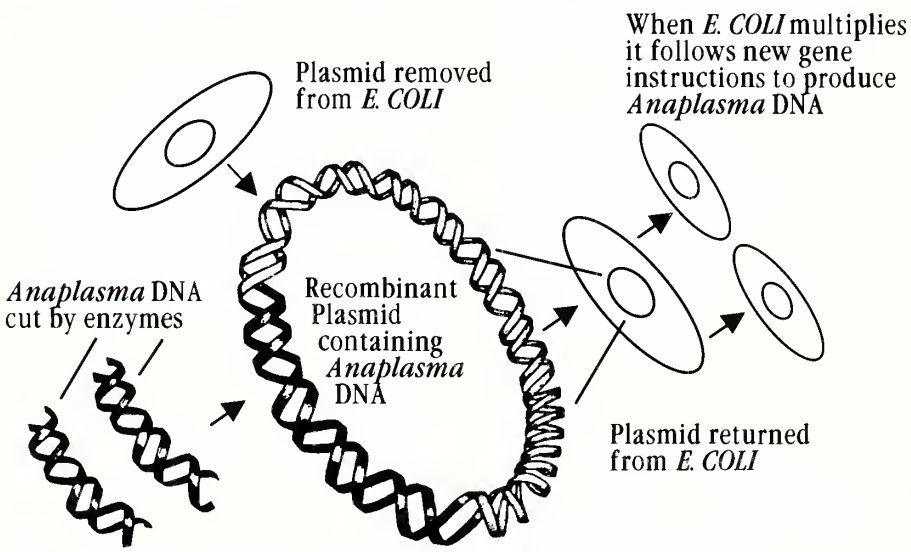
DNA is the source of all the excitement. I will give you a quick rundown on this nucleic acid. I am sure that any biochemist would be appalled, but we are not in a classroom. DNA is the stuff found in the cell's chromosomes and resembles a twisted zipper. It contains all of the information to make all of the proteins needed to end up with the distemper virus or a horse. The ribbon-like strands of DNA are held together by the teeth. The teeth of the zipper consist of four subunits called nucleotide bases (A,T,G,C). I won't go into this now—but most of the "business" of DNA's replication is carried on with the teeth. When DNA is unzipped, each side acts as a pattern and another zipper of DNA is formed. A gene is a piece of DNA—really a blueprint for a protein.

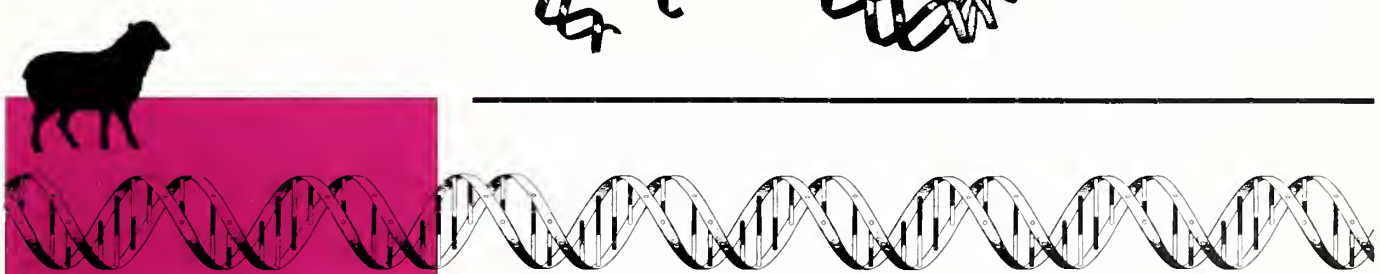
Although the DNA zipper (double helix) was first described in 1953, DNA wasn't an everyday term to many of us until the early 1970's, when we read in the daily newspapers that insulin could be manufactured in *E. coli* Briefly, the technique which provided the basis for recombining DNA is as follows: The bacterium *E. coli* contains several small rings of genetic material called plasmids. The plasmids are soaked in an enzyme causing them to open at a specific place on the ring. A gene is snipped from a segment of DNA that comes from a completely foreign source such as the gene for insulin. The foreign insulin gene is spliced into the *E. coli* plasmids—thus forming *recombinant DNA*, that is, DNA from two different organisms. The *E. coli* bacteria take the new gene instructions and make insulin. Anaplasma DNA is used to illustrate the recombinant DNA procedure in the figure 1.

When *E. coli* were turned into "insulin factories", it was immediately apparent that recombinant DNA technology might provide more effective and less expensive vaccines and other medically significant proteins. In the seventies and eighties, genetic engineers were unleashed in commercial, government, and university laboratories to make, modify, and move DNA around in all sorts of experiments. The race is on, and biotechnology companies appeared overnight.

After insulin, recombinant human and bovine growth hormones were produced. We have all read how

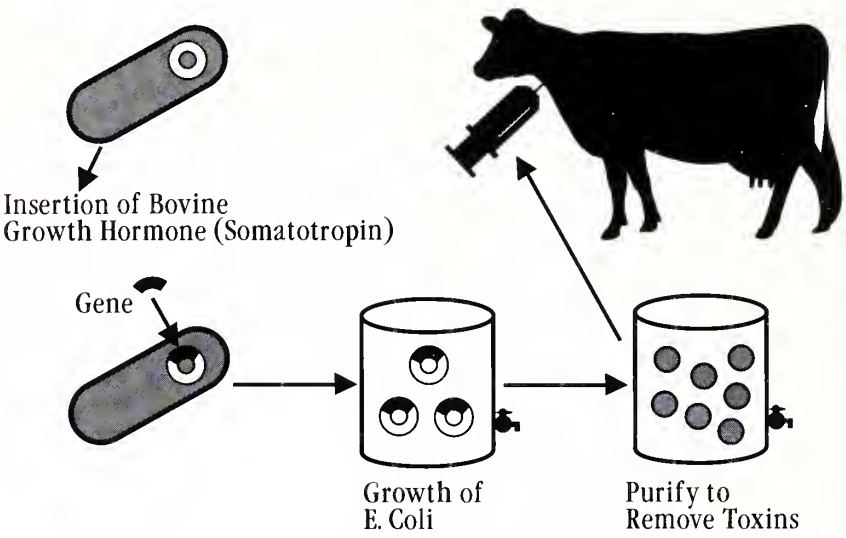
Figure 1. Anaplasma DNA Illustrates the Recombinant DNA Procedure.





athletes have used human growth hormones to their own detriment, but human growth hormones have helped about 3,000 human dwarfs each year. The use of bovine growth hormone (bovine somatotropin—BST) is causing a great deal of controversy. When injected into cattle, BST will increase their milk yield by 10-25 percent. On the other hand, dairy practitioners must realize that there is a possibility that a percentage of our Nation's dairy herd may be lost. We need to know more about the long-term effects of BST on the cow. Will dairy practitioners see more metabolic disease and mastitis in these higher producing cows? Then there are consumer concerns, even though there is no research that suggests that BST is dangerous for humans.

Figure 2. Production of Bovine Growth Hormone (Somatotropin).



High-Tech Vaccines

Genentech and the USDA tricked *E. coli* into making a foot-and-mouth protein for use in a vaccine. However, this genetically engineered vaccine is more expensive to make and not as effective as the present foot-and-mouth disease inactivated vaccines. There are more than 50 foot-and-mouth virus subtypes, and many of these show little cross-protection against each other. The cloning of virus types for F-and-M vaccines would be a monstrous job. On the other hand, the search goes on to find an antigen that's common to all F-and-M types.

A synthetic vaccine is another example of a "high tech" vaccine. On the surface of viruses, there are proteins (peptides) that stimulate an animal to produce antibodies and cellular resistance. Laboratory workers have "mimicked" these proteins in the case of foot-and-mouth disease and some human viruses. They took amino acids off the shelf, mixed them together, and made vaccines. But synthetic vaccines were not as effective as present-day "inactivated vaccines."

Many researchers feel that vaccinia vectored vaccines are the real hope of future bioengineered vaccines. Research workers have inserted the gene or genes of foreign disease agents into the vaccinia virus. Workers at the University of California at Davis have prepared a successful vaccine against vesicular stomatitis and rinderpest viruses.

Molecular virologists have taken the pseudorabies virus apart and removed the thymidine kinase (TK) gene. Without thymidine kinase the vaccine cannot multiply in the central nervous system of pigs and establish latency. The gene deletion vaccine has attributes making it superior to inactivated virus pseudorabies vaccines and also conventional live-virus pseudorabies vaccines. Also, deletions allow serologic differentiation between vaccinated pigs and those infected by a wild field virus.

DNA (Gene) Probes

The diagnosis of disease took a giant step forward with the advent of DNA probes (also termed gene or nucleic acid probes). When DNA is "unzipped" into two strands, each strand will only match up and bind with its complementary nucleotide bases. A DNA probe is a segment of one DNA strand with a radioactive or other signal that is used to search the tissues of an infected animal or vector such as a tick for viral or bacterial genes. A probe can also identify a defective

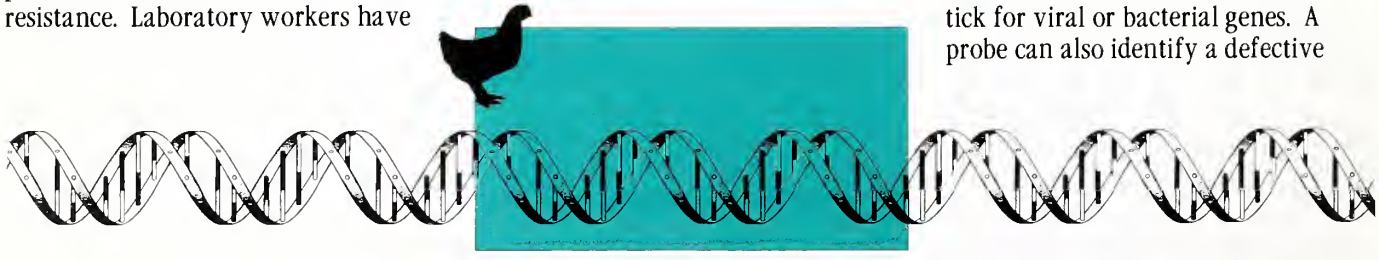
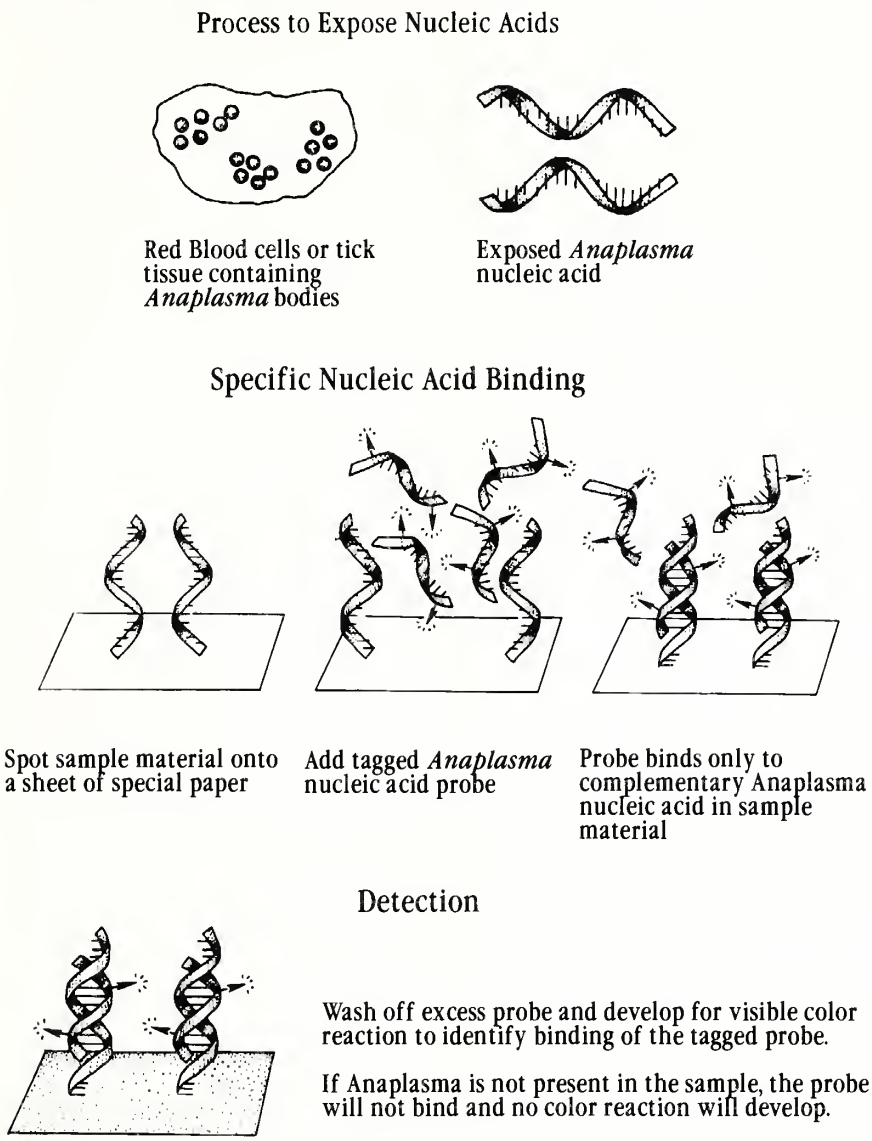


Figure 3. Nucleic Acid Probe Test for Detecting Anaplasma marginale.



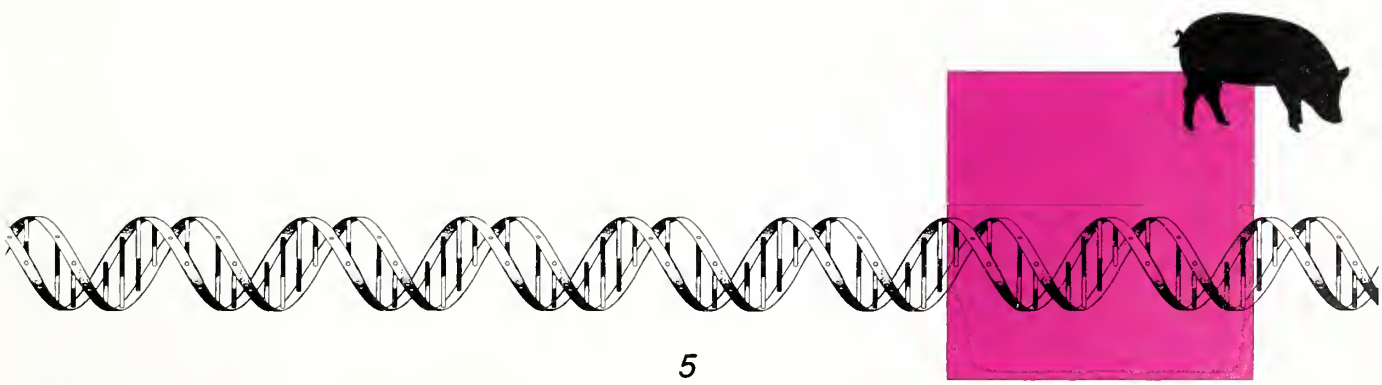
gene in case of a hereditary disease. DNA probes offer unprecedented sensitivity and specificity for diagnosis. The figure 3 shows the use of an anaplasma DNA probe to detect infected red blood cells (RBC/s) on ticks.

Researchers are developing DNA probes for such diseases as malignant catarrhal fever, pseudorabies, infectious bovine rhinotracheitis, bluetongue, bovine virus diarrhea, transmissible gastroenteritis, ovine foot rot, brucellosis, colibacillosis, mycoplasmosis, tuberculosis, swine dysentery, Johne's disease, leptospirosis, distemper, and anaplasmosis.

Swine dysentery, leptospirosis of cattle and swine, and Johne's disease of cattle establish chronic carrier states and perpetuate disease by excreting bacteria into their environment. Thus, detection of carrier animals is essential for controlling these diseases. Conventional detection methods for diagnosing these three diseases are inadequate because the bacteria that cause them are particularly difficult to isolate and grow very slowly in the laboratory.

Transgenic Animals

Human beings have been improving the genetic quality and productivity of livestock for thousands of years. This improvement has been painfully slow because the selection and breeding for desirable traits had to be based on the genes already present in the breed.



In the future, classical breeding methods may be bypassed by microinjecting desirable foreign genes directly into one-cell embryos. Animals carrying new genes are called transgenic. The new gene expressing a desirable trait will be passed on to the offspring in the same way as the animal's own genes.

Scientists have shown that transgenic animals will have a major impact on the livestock industry of the future. The long-term goal is not to produce giant cows or pigs; their experiments are designed to introduce economically important genes into livestock. Livestock producers and the consumer will benefit from the development of meat animals that utilize feed more efficiently, have an enhanced rate of gain, have increased resistance to diseases and parasites, and produce a leaner product.

Molecular Farming

British molecular biologists took genes that code for desirable therapeutic proteins and injected them into one-cell embryos of a ewe. Then these embryos were implanted into a surrogate ewe, and when the female lamb was born and matured, it was found that milk from that sheep contained the desired protein. While the first work was with the human factor IX, an antihemophilic substance, it immediately suggested that medically important proteins could be obtained in relatively large quantities in the milk or blood of transgenic dairy animals.

Why Do Some Animals Get a Disease, While Others Do Not?

Geneticists have tried to link genetic traits with disease-susceptibility without a great deal of success. Until recently, they simply have not had the tools. Both in humans and in animals, there are clusters of genes that an animal inherits from its parents that are associated with susceptibility and resistance. Through the use of monoclonal antibodies and recombinant DNA technology, researchers have been able to detect antigenic markers on lymphocytes which can be correlated with disease resistance and/or susceptibility. Trichinosis, bovine leukemia virus, and scrapie are three diseases that are under significant genetic control.

What Will Biotechnology Mean to the Practitioner?

Even though genetic engineering technology is advancing at a very rapid rate, and there are many potential applications, there are but a few practical uses today. However, with recombinant DNA, DNA probes, and the micromanipulation of embryos to make transgenic animals, we are entering into a decade of spectacular basic and applied research that will have a profound impact on our profession.

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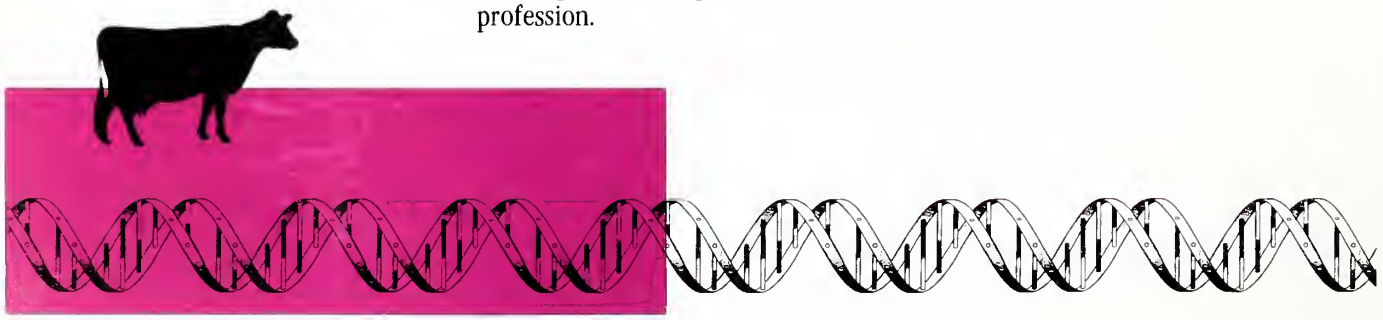

Impact on Immunodiagnostics

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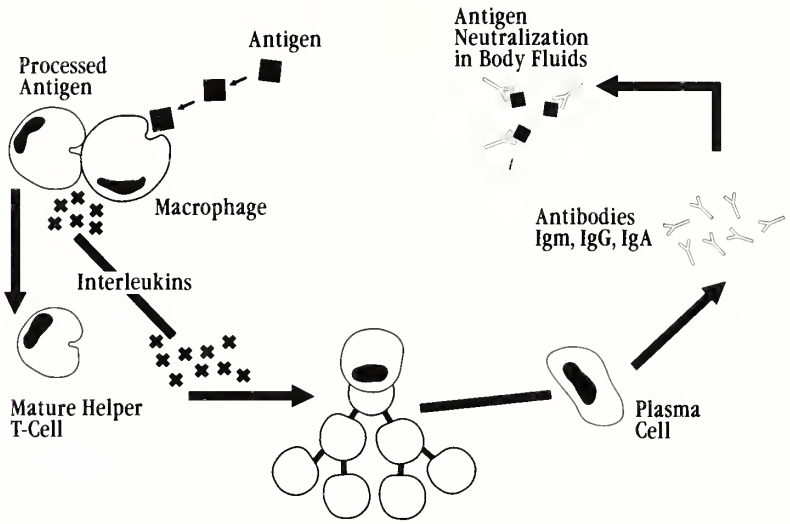
Biotechnology has been applied for thousands of years by those who utilize micro-organisms for the production of foods and beverages. During the last century, micro-organisms have been used for the production of medically important substances such as antibiotics to help control disease in man and animals. Over the last 30 to 40 years, advances in biotechnology have led to advances in plant hybridization and animal reproduction including embryo transplants and superovulation.

In more recent times, biotechnology has come to be identified with three major disciplines: bioengineering, cell biology, and molecular biology. With respect to bioengineering, advances in solid phase chemistry, separation technology, sensor technology, and instrumentation have made practical both the production of biological reagents and the marriage of biologicals with sophisticated instrumentation and disposable devices. Research in cell biology and advances in immunology have generated techniques for fusing lymphocytes with cancer cells producing hybrid, immortal cell lines or hybridomas. Such cell lines produce antibodies which are called monoclonal antibodies. In terms of molecular biology, the development of recombinant DNA techniques has allowed scientists to insert DNA segments into the genomes of bacteria, yeast, and mammalian cells. Subsequently, the altered host organism produces the protein encoded by the recombined segment.

Figure 1. Simplistic diagram of the immune system.

The Immune Response

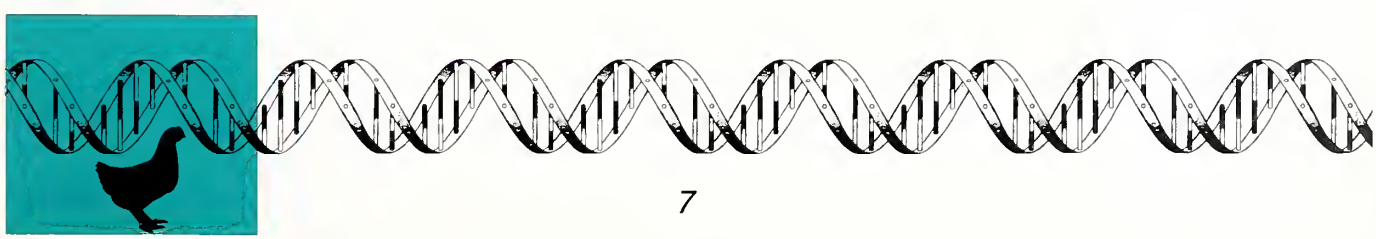


The diagnosis of disease is among the first areas of medicine to be strongly influenced by modern biotechnology. This presentation will describe the impact of biotechnology on immunodiagnostics as applied to the diagnosis of disease in animals.

Immunoassay Technology

Immunodiagnostic tests are playing an increasingly important role in the diagnosis of disease. Tests that once could be done only in reference laboratories can now be performed in the veterinarian's office. The use of new assay delivery systems incorporating monoclonal antibodies and recombinant antigens have given rise to improvements in sensitivity, specificity, and ease of use.

Immune Response. Before discussing immunoassays and monoclonal antibodies, it is useful to briefly review the immune response. As shown in figure 1, when an organism is challenged with viral or bacterial antigens, this "foreign material" is first processed by the macrophages. This involves breaking up the material into fragments which are presented in association with the major histocompatibility complexes to the T-lymphocytes (T-cells)[1]. The T-cells are subsequently activated and stimulated to divide. Note that the interleukins play a role in T-cell activation. B-lymphocytes (B-cells) also interact with the foreign antigens as a function of receptors different from those on T-cells. B-cell receptors are known as immunoglobulins. Subsequent to an interaction with antigen, activation of the B-cells is completed via stimulation by other



lymphokines secreted by the T-cells. The activated B-cells then undergo cell division producing B-cell clones. Each clone is capable of producing a specific immunoglobulin recognizing one site or epitope on the viral or bacterial antigen. These B-cell clones mature into plasma cells whose function is to produce and secrete immunoglobulins into the blood. These antibodies circulate throughout the body and are capable of binding to foreign antigens. This binding triggers additional mechanisms which process and neutralize the foreign antigen[2].

Antibodies are developed against a variety of epitopes on the viral or bacterial antigens. The ability of antibodies to recognize subtle differences between two very similar protein molecules is important for the development of highly specific diagnostic tests.

Given the characteristics of the immune response and the nature of the interaction between antibodies and antigens, we are able to utilize the immune system in two ways. First, diagnostic reagents, that is, polyclonal and monoclonal antibodies, may be produced in a variety of host animals. Second, the antibodies produced by an animal subsequent to infection or artificial immunization by a disease organism may serve as a marker for that challenge.

Immunoassay Design

There are three basic requirements for an immunoassay. First, an appropriate source of antibody or antigen is required. Typically, if the assay is confirmed to detect antigens, antibody is required; if the detection of specific antibody is the goal, then a source of purified viral or bacterial antigen is needed. Next, a matrix in or on which the antibody/antigen reactions occur must be available. Finally, a readout system to measure the extent of the reactions is required. Utilization of these basic criteria allows the development of a great variety of test configurations.

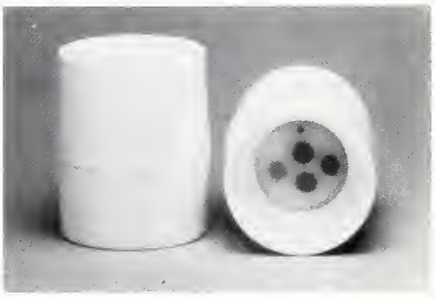
Immunoassay systems have evolved dramatically over the last several years from the early systems which included simple agglutination tests, coated tube tests, and coated bead tests to sophisticated, automated sample-in-answer-out systems as well as quick, easy-to-use, and accurate in-field or in-clinic systems. All systems incorporate the three aforementioned basic requirements. Such advances in immunoassay technology have been applied to laboratory and in-field testing for a number of animal diseases including brucellosis. The PCFIA (Particle Concentration Fluorescence Immunoassay) test system as shown in figure 2 is a fluorescence-based automated immunoassay which provides sample-in-answer-out capability to the laboratory for brucellosis testing at a rate of 550 samples per hour. Also shown in figure 2 is an in-field brucellosis test using membrane filter technology. This test may be performed in less

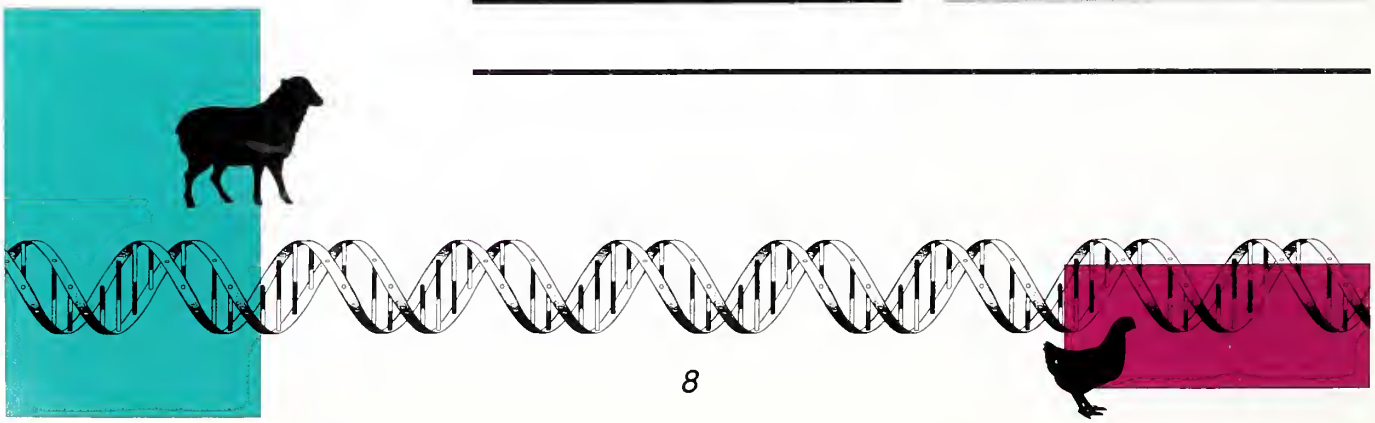
Figure 2. Examples of the evolution of immunoassay technology: laboratory automation/fast accurate in-field tests.

Complementary Field and Lab Tests

Laboratory

Field-In Clinic





than 10 minutes with sensitivity and specificity essentially equivalent to the PCFIA system. Furthermore, this technology has been applied to the diagnosis of a number of companion animal diseases and is used extensively in veterinary clinics around the world.

Impact of Monoclonal Antibodies on Immunoassay Design/Function

In addition to advances in bioengineering, monoclonal antibody technology has also had a dramatic impact on our ability to design more practical immunoassays and better diagnose disease.

A schematic diagram of monoclonal antibody production is presented in figure 3. The practical advantages of monoclonal antibodies are evident in that stable hybridomas can produce large quantities of antibody with unchanging affinity and specificity for the antigen of interest[3]. From a quality control or a manufacturing point of view, the use of monoclonal antibodies in immunodiagnostic tests is desirable. Furthermore, from a functional perspective, the use of monoclonal antibodies can provide additional benefits in terms of specificity and ease of use.

Monoclonal antibodies are particularly useful in competitive assays designed to measure specific antibody levels in test samples. Such an assay is depicted schematically in figure 4. In this case, the objective was to construct an assay that would differentiate a vaccinated pig from an animal infected with

Figure 3. Production of monoclonal antibodies.

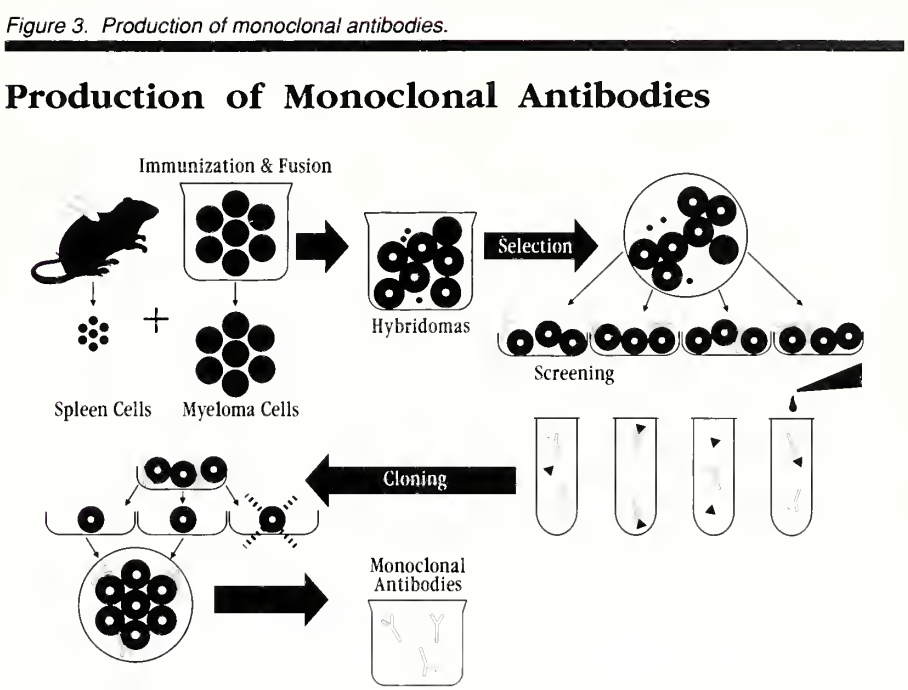
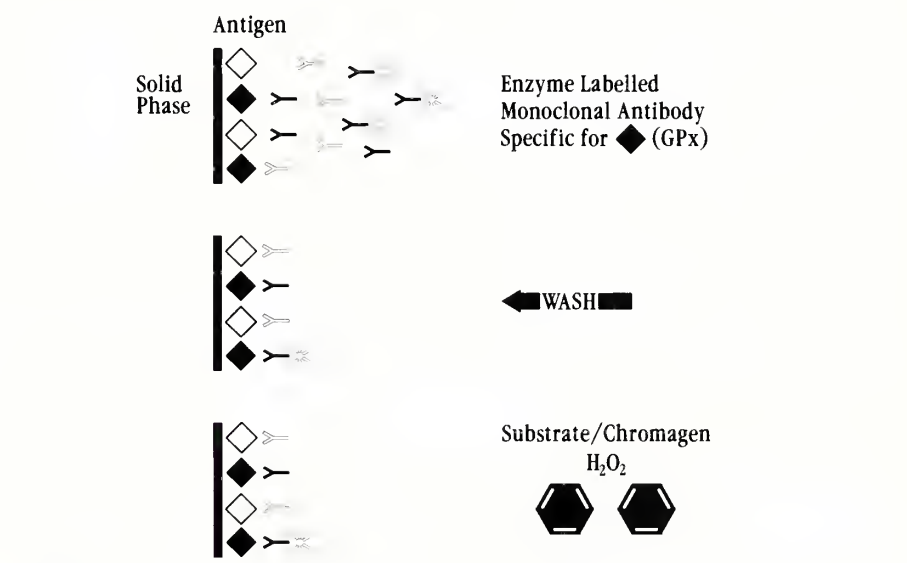


Figure 4. Monoclonal antibody-based competitive ELISA for the detection of specific antibody.



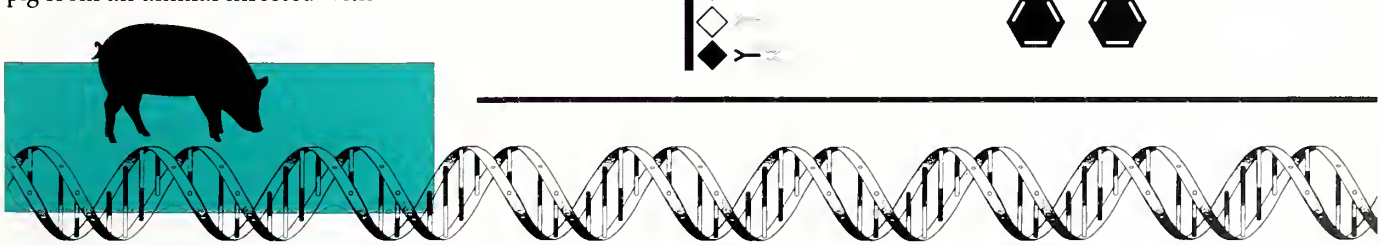
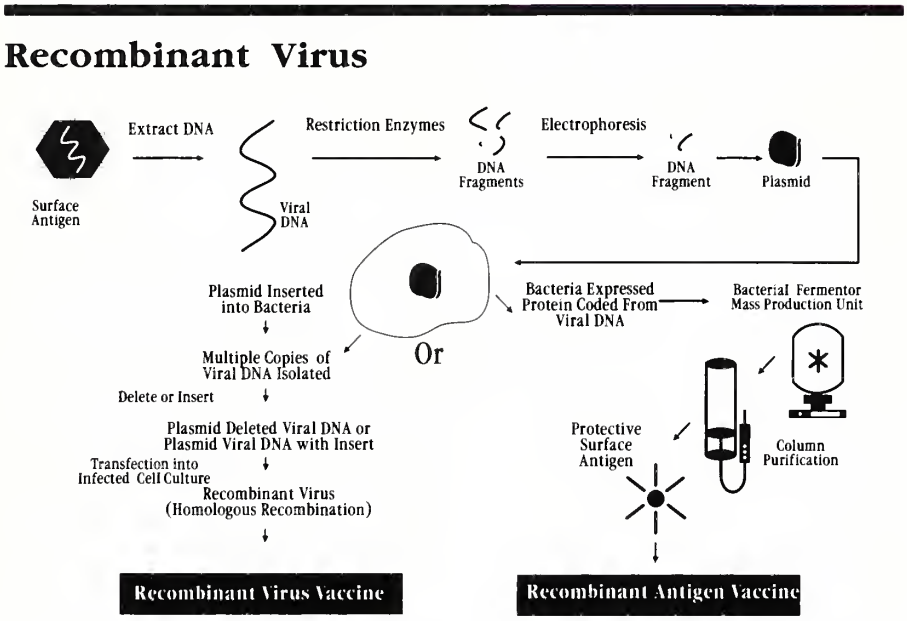
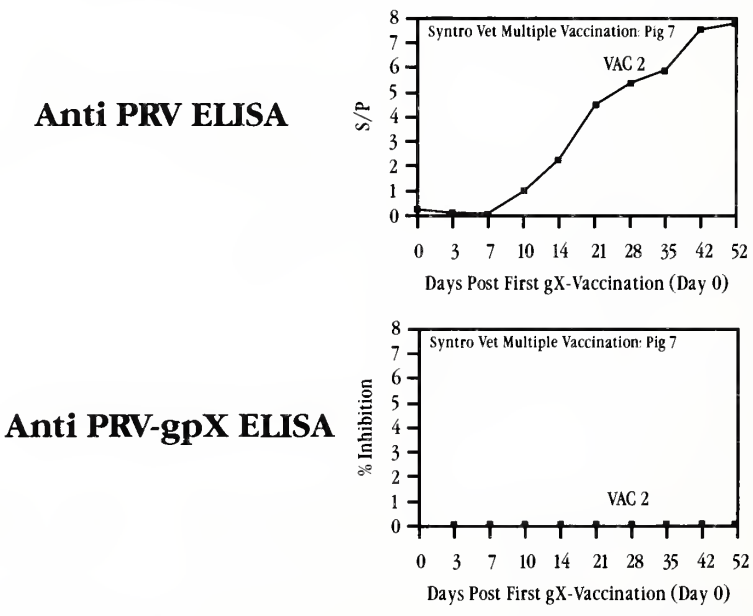


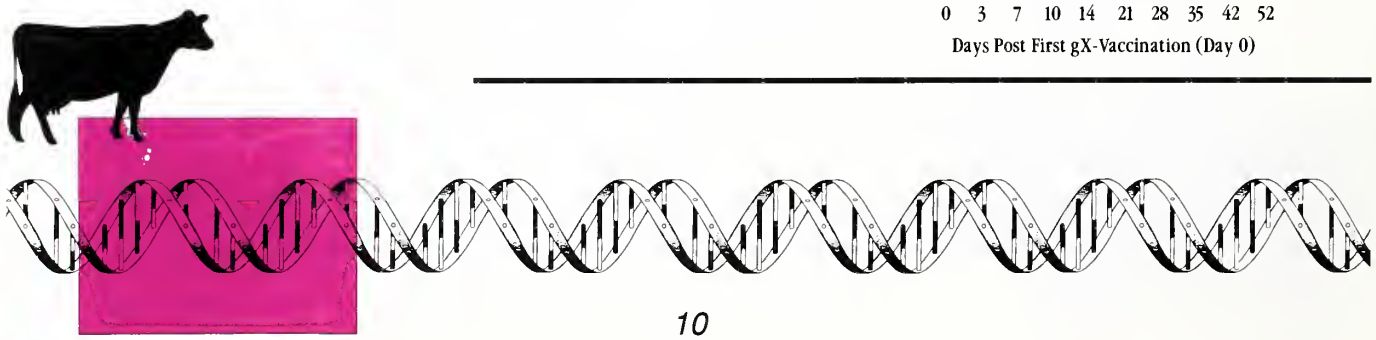
Figure 5. Schematic of recombinant DNA technology.



Construction of an assay for gpX required that monoclonal antibodies be developed against this glycoprotein. A suitable clone was selected and labeled with enzyme. Crude viral antigens could be utilized on the solid phase, since the specificity of the assay is determined by the monoclonal antibody. In this assay system, antibodies in the test sample specific for gpX compete with the labeled monoclonal antibody for binding sites on gpX. Results of such an assay are shown in figure 6. The level of sensitivity of the gpX assay is the same as the current screening tests on wild type infections, yet shows no response on animals vaccinated with a gpX-deleted vaccine. Such a monoclonal antibody-based assay in combination with engineered vaccines should make "wild type" pseudorabies virus (PRV). The vaccine strain of this pseudorabies virus was developed using recombinant DNA technology (figure 5). Genes coding for an enzyme required for the virus to replicate in nerve cells as well as viral glycoprotein (gpX) were deleted from the viral genome. Consequently, vaccinated animals produce antibodies to all other viral glycoprotein components whereas swine exposed to the wild type virus produce antibodies to all viral components including gpX. Thus, the use of a gpX-deleted vaccine and an assay specific for antibodies to gpX allow differentiation of vaccinated and infected animals.

Figure 6. Top Panel: Anti-PRV ELISA on 2x vaccinated animal; Bottom Panel: Anti-PRV gpX ELISA on 2x vaccinated animal.





a significant contribution to the eradication of PRV around the world.

Utilizing monoclonal antibodies in the assay system presented in figure 7 can simplify the assay protocol. A diagram of a feline leukemia p27 antigen test is presented in figure 8. Monoclonal antibodies on the solid phase are directed against a different epitope than the enzyme labeled monoclonal antibodies. Thus, the sample containing the FeLV antigen may be incubated simultaneously with both solid phase antibody and labeled antibody without competition between the two. In contrast, when using polyclonal antibodies, two incubation steps are required instead of one incubation.

Molecular Biology

Recent advances in the understanding of the structure, function, and regulation of genes now allow manipulation of biological systems such that cloned DNA may be inserted into bacteria, yeast, or mammalian cells, allowing expression of the proteins coded for by that DNA. The resulting material is often in abundant supply, relatively inexpensive to produce, and may be used effectively in various diagnostic test formats. In addition to the expression of important antigens, current technology allows one to obtain specific nucleotide sequences from mixtures of DNA molecules. This, of course, is the basis of DNA probe technology which can be an important tool in the diagnosis of disease.

Figure 7. Schematic of a typical antigen detection assay.

ELISA Technology

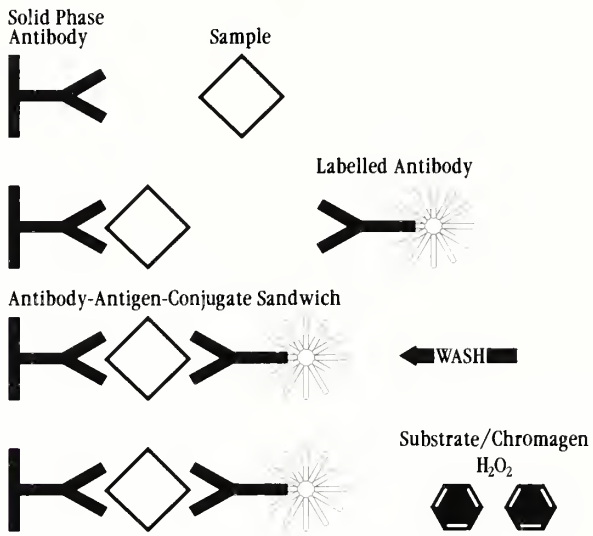
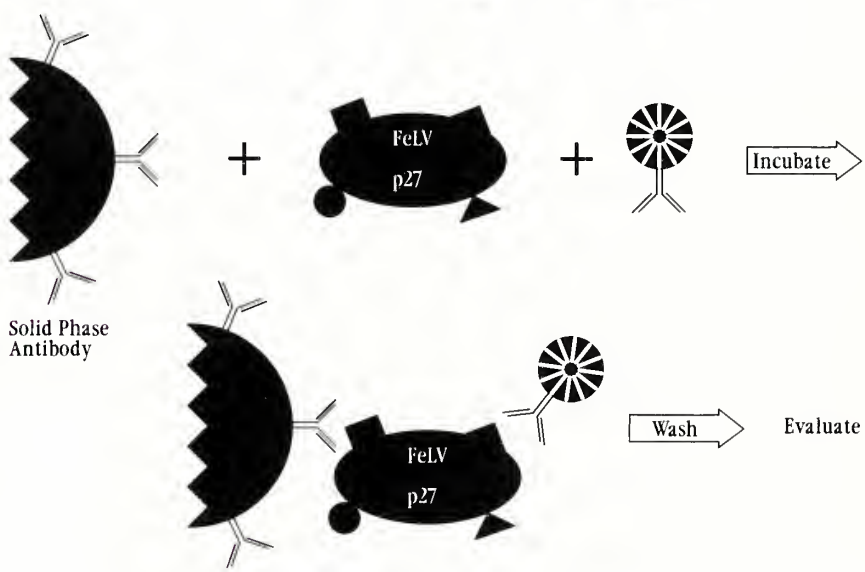
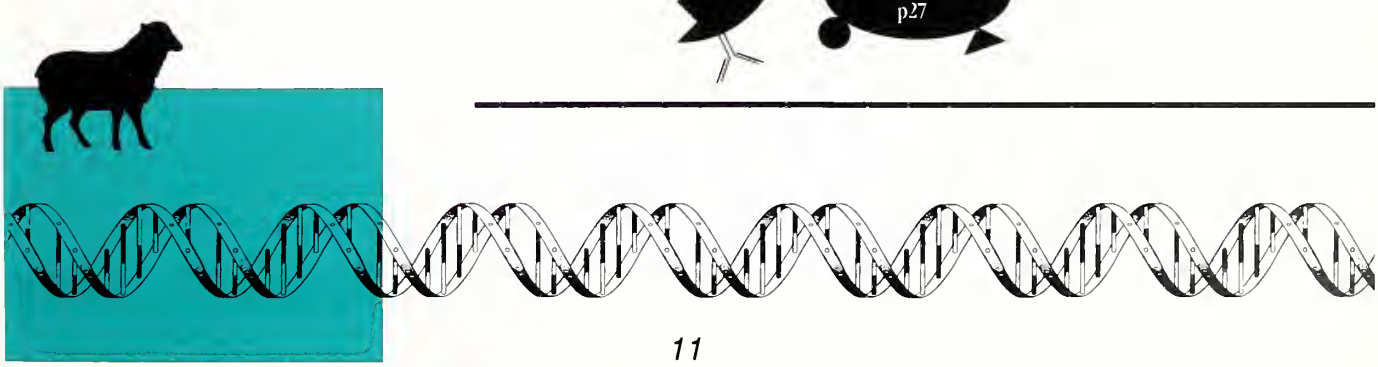


Figure 8. Simultaneous incubation of p27 antigen with monoclonals recognizing different epitopes.





Utilization of Recombinant Antigens in Immunoassays.
Assays designed to detect antibody as a marker for infection require a source of antigen specific to the particular infectious agent of interest. Oftentimes, the production of virus or bacteria as a source of this antigen is difficult and very expensive. Examples include production of human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV), both of which are relatively difficult to produce in tissue culture.

In the case of HIV, several assays have been constructed utilizing cloned viral proteins expressed in *E. coli,* yeast, or mammalian cells. Of principal importance in HIV and FIV diagnostic test designs is the incorporation of specific envelope and core proteins to provide sensitivity and specificity equivalent to or greater than the whole virus assays currently available. In the near future, a number of immunodiagnostics will be available to veterinarians which incorporate recombinant antigens and provide better sensitivity and specificity at a lower cost.

Summary
The evolution of immunodiagnostic technologies driven in part by advances in biotechnology has had and will continue to have a very positive effect on our ability to diagnose disease. Automated immunoassay systems have been introduced for high volume testing applications, providing sample-in-answer-out capability with improved accuracy. Improvements in solid phase chemistry and novel applications of monoclonal antibody technology have produced extremely accurate, rapid, and easy-to-use tests for feline leukemia virus, FIV, canine heartworm, canine parvovirus, and brucellosis for in-clinic and in-field use reflecting. Monoclonal antibody-based tests allow veterinarians to distinguish vaccinated animals from animals infected with the pseudorabies virus. In the near future, the use of recombinant antigens in immunoassays will provide more accurate and less costly tests. Without a doubt, veterinarians have benefited from the application of biotechnology to veterinary diagnostics in terms of their ability to deliver high-quality, rapid, and cost-effective health care.

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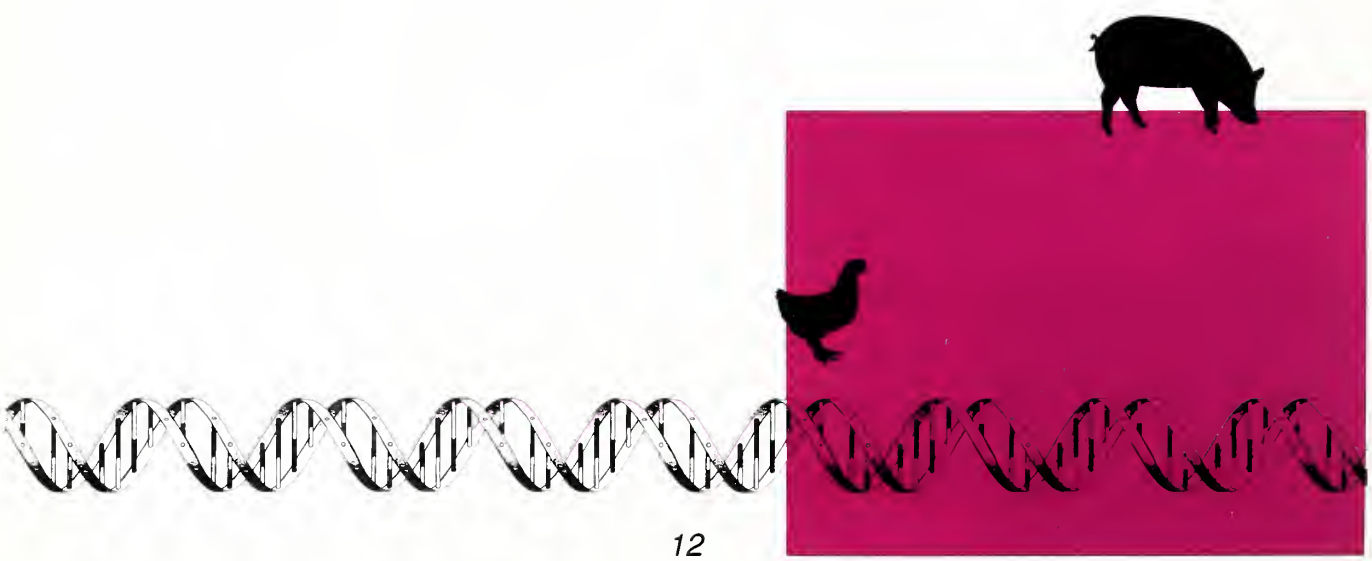

Probes To Identify Disease

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Abstract: The recent advances in biotechnology are having a major impact on the diagnosis of disease in veterinary medicine. The application of enzyme linked colorimetric assays, the use of nucleic acid probes to identify genetic material from microorganisms or animals, and immunoassays to identify subunit proteins of microorganisms have brought highly sensitive, specific, and rapid diagnostic procedures to veterinary medicine. Technology is advancing in some areas to the point that animal-side tests are now available. The fact that much of this technology can be automated now makes it possible to monitor diseases more rapidly and extensively than was the case in the past. Molecular epidemiology is permitting the tracing of organisms to the farm of origin and in some instances to the animal of origin. It is also possible to discriminate vaccinated from naturally infected animals. In the future it may be possible to identify those animals that may be either susceptible or resistant to disease.

Introduction

The traditional methods of diagnosing disease include clinical signs, pathological lesions, isolation of the causative agent, and/or serological test procedures[1]. Many times clinical signs may be nonspecific as a number of different infectious agents may cause similar signs. Similarly, a set of lesions may be associated with the pathology of a number of diseases. Clinical and pathological parameters require the full expression of the disease. Subclinical forms of the disease or infection are usually overlooked in these situations. Isolation of organisms can occur within 24 hours or as long as 4 to 6 months of culturing. In many instances, the methods currently in place are labor intensive. The most commonly used technique for confirmatory diagnosis is serology. This procedure indicates that an animal has been exposed to an agent; however, it is not an indication that the agent is the cause of the disease at that time.

The recent advances in molecular biology and biotechnology are impacting the area of diagnostics in both human and veterinary medicine. Specifically, the use of radiolabeled or enzymatically labeled markers makes it possible to identify with great sensitivity molecular markers in tissues, in solutions or on solid matrices. These markers can be attached to nucleic acids or to antibodies that are used to identify genetic material or subunit antigens on microorganisms or for specific chemicals. Cloning of genes provides a means of rapidly propagating large quantities of specific nucleic acids which can be used as standardized reagents for diagnostic procedures. Similarly, monoclonal antibodies are highly specific reagents which are directed to specific epitopes on microbial agents or to specific chemical structures associated with toxic principles. Examples of the applications of these reagents to animals will be the subject of this manuscript.

Detection Systems

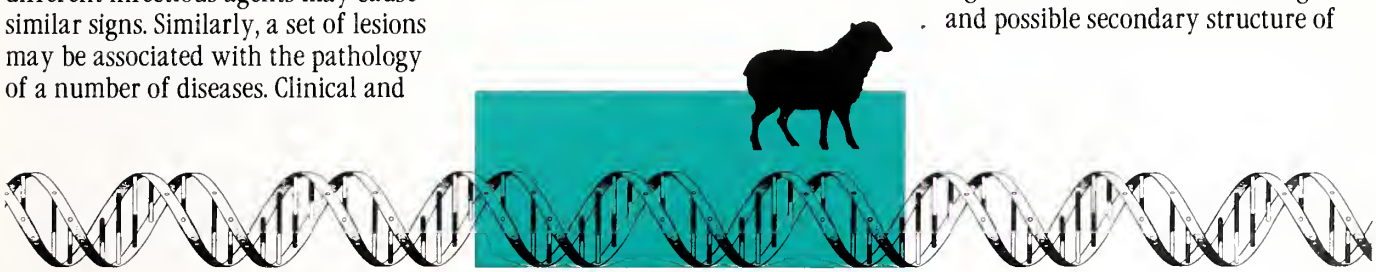
Major advances in detection systems have had a significant impact on rapid diagnostic technology[2]. The principle detection systems in use today are the radioactive/nonradioactive and direct/indirect detection systems. These detection systems consist of signal-generating molecules which are linked to ligand-specific biomolecular probes such as nucleic acids or antibodies. The enzyme/radiolabeled molecules offer the means for evaluating the result of an assay. Most detection systems are multipurpose, that is, they can be used on more than one probe. The specificity of the response lies with the nucleic acid or antibody probe.

Molecular Biochemistry and Diagnosis

Modern molecular biochemistry is proving helpful in providing more rapid and sensitive diagnostic techniques to detect infections in domestic animals. These techniques do not rely on the isolation and identification of a live replicating microorganism, but on the detection of highly specific molecular subunits of the inciting agent in tissues. These subunits may be either gene sequences specific for the microbe or specific protein epitopes on the protein components of the microorganism.

Biochemical structures

Polyacrylimide gels (PAGE) have made it possible to separate genomic and subunit proteins by molecular weight and charge. The migration distance in a gel is related to molecular weight and possible secondary structure of



the genomic segments[3]. Genomic segments may be obtained from microorganisms by enzymatic digestion, by restriction endonucleases, or in the case of reoviruses, by genomic disssociation at the time the viruses are released from the nucleocapsid. This technique is a crude means of fingerprinting; however, it has proven very useful for determining biotypic characteristics of certain viruses and bacteria. For instance, the technique has been used to demonstrate reassorting gene segments in bluetongue viruses and to differentiate reproductive from respiratory strains of bovine herpes viruses[4,5]. This procedure is also used to identify plasmids that may transmit virulence or antibiotic resistance factors in bacteria[6]. PAGE is also used to separate microbial proteins. Again, the subunit proteins are separated based upon molecular weight, conformation and charge. This procedure permits one to determine the number of subunits associated with the microbe and their functional activities[1].

Molecular Cloning of Microbial Genomic Material

Cloning of genetic material provides a means of propagating large quantities of a single gene that can be used for diagnostic purposes. Individual gene sequences can be isolated on PAGE cut out of the gel, eluted, copied and then spliced into a bacterial plasmid[1]. The plasmid is then exposed to bacteria. The bacteria with the plasmid incorporated into it undergoes replication. As the replication occurs, there is also replication of the foreign genes. Since bacteria replicate rapidly, it is possible to obtain large quantities of the isolated gene as well. The copied foreign gene can then be extracted from the plasmid and used for diagnostic purposes.

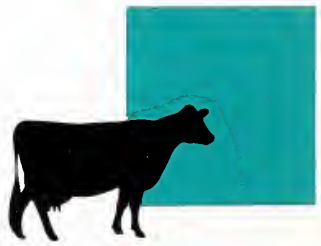
Genetic Probes as Diagnostic Tools

Cloned cDNA copies of individual genome segments are exact replicas of the original genomic segment from which they had been cloned. Genetic strands with complementary nucleotide sequences will hybridize to gene strands with similar nucleotide sequences. Hybridization can occur between complimentary DNA or RNA genomic materials[7,8]. Hybridization conditions for DNA/DNA and DNA/RNA genetic hybridizations on solid supports have been standardized.

Molecular Genetic Diagnostic Tests

The cDNA probes can be used in four diagnostic tests, Northern and Southern blots, dot blot hybridization and *in situ* hybridization. Northern blots can be used to identify RNA in cDNA/RNA hybridization assays. The most common use is for RNA viruses. Southern blots can be used for DNA in viruses or bacteria. Northern and Southern blots are performed following the placing of the test sample on PAGE and then electrophoretically transferring the genetic material to a solid matrix. The cDNA-labeled probe is administered to the matrix and if the complementary nucleic acids are present, hybridization occurs and a signal appears on the matrix. Dot blots are performed by extracting the nucleic acids from tissues and applying them directly to the solid matrix. The labeled probe is then administered and if the complimentary nucleic acid sequences are present, hybridization will occur. *In situ* hybridization utilizes the labeled probe by applying it directly to tissues. The probe will seek out complimentary nucleic acid sequences and hybridize to it within cells or tissues on glass slides.

A relatively new procedure known as the polymerase chain reaction (PCR) offers great promise for expediting the time required for a diagnosis[9]. The principle of the procedure is to use an oligonucleotide primer from a constant region of a gene. Extracted nucleic acid from tissue is mixed with the oligonucleotide primer. The primer will anneal to complimentary nucleic acid of the microbe and then the bound nucleic acids are placed in a thermocycler along with a polymerase enzyme. The thermocycler will alternatively heat to 90°C and then cool to 30°C. This difference in temperature is important for separating the annealed nucleic acids and to create new copies of the gene through the action of the polymerase. The result is a rapid amplification of the specific nucleic acid which can then be



used for diagnostic purposes[2]. A single virus can be identified in a sample of blood by using this amplification system[10].

Molecular Diagnosis of Specific Microbial Antigens

Another approach to modern molecular diagnosis involves the immunological identification of specific epitopes on proteins.

Protein Dot Blot Assay

The assay involves dotting 1-2 ul of monoclonal antibodies onto nitrocellulose strips. After air drying and blocking with gelatin, supernatants from microbial cultures are incubated with the dots on the strips. The monoclonal antibody originally bound to the NC paper forms complexes with the specific protein in the test culture supernatant. A rabbit polyclonal antiserum is added to the test-enriched supernatant followed by a biotin-conjugated affinity-purified goat IgG antirabbit IgG. The presence of bound goat antirabbit is detected by an avidin-peroxidase conjugate with appropriate substrate. A positive color reaction is an indication of monclonal-specific antigens in the test culture supernatant.

***In situ* Hybridization Assay**

Indirect immunoperoxidase procedures using both polyclonal and monoclonal antibodies have been successfully applied to detect microbial proteins in formalin fixed tissues. After fixing in formalin or by rapid freezing, tissue sections are washed, blocking steps applied, and the tissues are incubated with antimicrobial specific antibodies.

Immunoblotting Diagnostic Test

Identification of microbial subunit proteins using a Western blotting procedure has been successful. Extraction of microbial proteins is then applied to a PAGE, which results in separation of the subunit proteins. The proteins are then electrophoretically transferred (Western-blotted) to NC paper. The strips of NC paper containing the separated microbial proteins are blocked with gelatin and incubated with polyclonal antiserum to the microbial agent. Biotinylated affinity-purified anti-immunoglobulin and avidin/peroxidase conjugate are added to the reaction. The microbial-specific proteins on the NC strip are visualized for a color reaction of the substrate.. Monoclonal antibodies against microbial-specific epitopes may detect the subunit proteins on Western blots or by immunoprecipitation.

Conclusion

Rapidly advancing technological procedures directed at subunit proteins as genetic material are increasing the specificity of diagnostic procedures. The use of novel marker systems which can be amplified through bridging molecules has increased the sensitivity of these systems. Many of these procedures are resulting in animal-side tests. This is greatly increasing the use of the tests, making it possible for veterinarians to make diagnosis in the field.

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Pseudorabies Virus Gene-Deleted Vaccines

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An integral component of our veterinary activities is disease prevention and control. The successful accomplishment of these activities will depend on good preventive measures to include the immunization of animals at risk. For the successful veterinarian, a firm background on vaccine characteristics and qualities is essential as is proper management of the products.

A good disease control program for pseudorabies must provide a means by which veterinarians and regulatory agencies can contain, prevent, and eventually eliminate the etiology of the disease. It must also provide a means by which the producer can maintain a successful livestock program and gain an adequate return from the investment. There must of necessity be an accord between veterinarians, regulatory medicine, and livestock producers such that it is beneficial for all parties to participate in disease control activities.

The past decade has spawned a host of new and novel approaches to the development of more unique and superior vaccines. These improvements are based on a better understanding of microbial pathogenesis and recent advances in genetics and molecular biology.

My task is to cover briefly the new generation of vaccines which are classed as Category II rDNA derived animal biologics [table 1][1]. Category II covers gene deletion/addition vaccines. I will also review current vaccines used for control of PRV and touch briefly on the prospects for future vaccines.

Table 1.

Categories of rDNA Produced Vaccines

Category I
  Nonreplicating vaccines
  rDNA-derived vaccines, bacterins;
  Bacterin-toxoids;
  Subunit viral or bacterial vaccines
Category II
  Live micro-organisms—
    with genes added or deleted
Category III
  Live subunit vaccines
  Live vectors carrying rDna
  Immunogenic genes or other
    immunizing antigens

History

Pseudorabies (PR) is an economically important disease of swine, caused by an Alphaherpesvirus [2]. Economic losses from PR vary with severity of clinical disease, age, and coexisting disease conditions [3-5]. Losses from PRV infection include: fetal death, reduction in breeding efficiency, decrease in growth performance, and in some areas, loss of options for sale of breeding stock and market pigs [6].

An important feature of PRV infections is the establishment of a latent state in which the viral genome becomes resident in ganglions of surviving swine [7]. This occult virus may be activated at various times, serving as a continuing source of infectious virus for susceptible pigs [8]. Latency of PRV is of critical importance to pseudorabies control. A thorough understanding of latency and its consequences is a must, if we are to effectively contain and eliminate PRV from infected domestic herds and feral pig reservoirs.

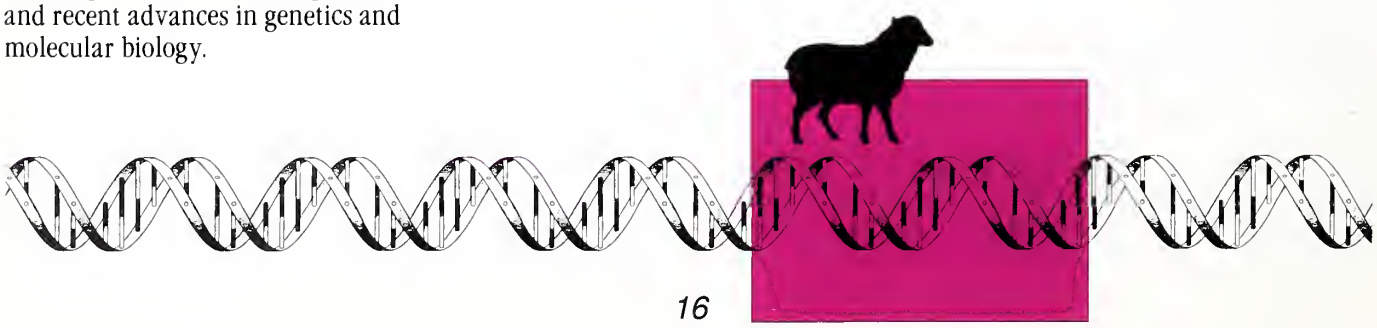
Table 1a.

PRV Eradication Programs

- Depopulation/Repopulation
- Test-and-Removal
- Offspring Segregation
- Vaccinatiion (alone)
- Combination of above

A number of programs for containment and control of PRV in infected swine herds exist [9]. They are shown in table 1a. Vaccination has been used as an important component of many eradication plans. The choice of the vaccine and how it is used is one of your most important decisions, and is critical to the success of the control program.

With few exceptions, the licensed vaccines for use in animals are produced by conventional methods (the term conventional refers to



methods that have been in use for many years). They include vaccines containing nonreplicating antigens (inactivated), and vaccines containing replicating antigens (live attenuated). Advantages and disadvantages of these products have been discussed elsewhere [10, 11, 12] and are well known to each of us.

Conventional vaccines have drawbacks that relate primarily to safety and efficacy [12]. Nevertheless, it has been through the use of these vaccines that many diseases have been brought under control [11]. Killed and modified live virus PRV vaccines have been available for use in swine for many years. Vaccinated pigs are afforded protection from clinical disease by these products. They are not, however, protected from infection. Additionally, vaccinal responses to most of the vaccines cannot be differentiated from those elicited by natural exposure to field virus.

Vaccines

Biotechnology—[recombinant DNA (rDNA), genetic engineering]—has provided us with many approaches for vaccine production. It has provided means for understanding virulence and pathogenesis of disease. Additionally, it brings forward new strategies for genetically tailoring vaccines. Currently there are four vaccines in use produced by this technology. Other new vaccines will appear in the future, some of which are now undergoing field trials.

Before we discuss the new generation of vaccines, let's review conventional products. Two types of vaccines are in common use today: (a) nonreplicating vaccines, commonly referred to as "killed" vaccines, be they subunit or whole organisms and (b) replicating vaccines, known as modified live or attenuated organisms.

Replicating vaccines are generally considered the most effective. They more closely mimic the natural infection, multiplying within the host and inducing a longer duration of immunity. With conventional modified-live vaccines, attenuation has been troublesome to accomplish since the methods used are empirical in nature; e.g., passage in foreign hosts (either animals or cell lines), growth at reduced temperatures, and chemical or radiation mutations. With these procedures, mutants occur but the correct level of attenuation may not happen.

Nonreplicating vaccines are relatively stable under environmental conditions and therefore can be used under more adverse conditions. The big disadvantage is that they do not replicate within the host and larger amounts of antigen are required for injection to ensure adequate immunity. Killed vaccines produce a humoral immunity but the immunity produced is limited. For some diseases, however, they are the only kind of vaccine available.

As good as some of the conventional vaccines are, they have limitations (table 2). For a number of microbial and parasitic diseases, there are no effective vaccines. Many are needed. There continues to be a need to develop and produce better vaccines which are safer, more effective, and genetically stable.

The rDNA techniques are refinements of the conventional techniques of biotechnology (e.g., cross breeding, natural selection by passage in aberrant hosts, chemical/radiation mutation, etc.) used during the past several decades. These refinements permit sensitive and precise manipulations of genes which result in better characterized effects. New strategies for producing vaccines are shown in table 3.

The rDNA technology has provided us with a wealth of information on molecular structure and genome organization of many viruses. This knowledge has been applied to the development of "designer vaccines" for control of pseudorabies.

In response to the needs of industry and regulatory officials to produce a vaccine that allows for differentiation between vaccinated and infected animals, science and industry have responded with the development of four genetically engineered vaccines for PRV: Omnivac-PRV, PRV/Marker, Tolvid, and Omnimark-PRV. Additionally, rDNA techniques have shown some conventional vaccines to have a gene deletion which can possibly be used in a marker test.

Kit and others in developing a new modified-live PRV vaccine focused on reducing the virulence of an established vaccine strain of PRV. The objective was a vaccine that provided high levels of protection against disease and that is safe in all animals,

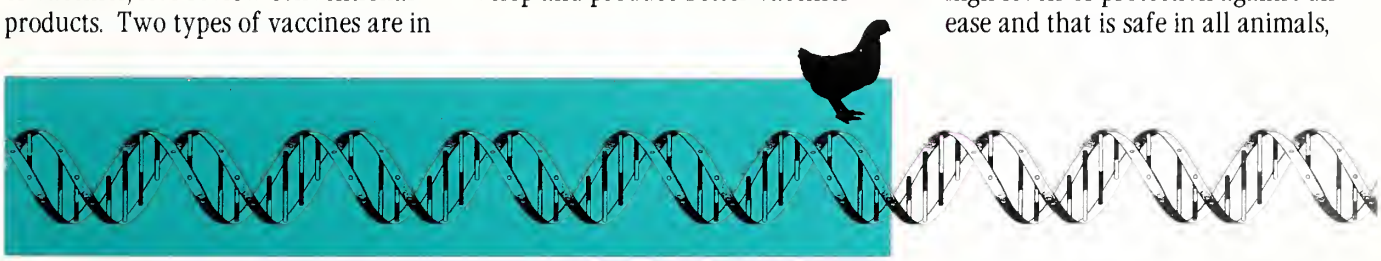


Table 2.

Disadvantages of Conventional Vaccines

- Potential for causing clinical disease
- Reversion to virulence
- Safety
- Complete inactivation of "killed" organisms essential but not always achieved in production lots.
- Adjuvants and multiple vaccinations required
- Side effects from presence of cellular material in some products
- Storage at temperatures essential for retaining infectivity and/or immunogenicity

including pregnant sows, newborn pigs, and heterologous animal species [13]. For this vaccine the tk gene was deleted.

The thymidine kinase (TK) gene of animal viruses was discovered over 30 years ago [14,15]. The tk produced by many herpesviruses shows a difference in enzyme substrate specificity from tk produced by the host cells. This difference in substrate specificity was used for developing selective antiherpes drugs [16]. Many tk-negative mutant herpes simplex viruses developed following prolonged therapy with iododeoxyuridine, Acyclovir, and other antiviral drugs.

Studies with drug-induced tk negative HSV mutants demonstrated a reduced virulence and an inability to establish latency [17]. It was proposed that tk negative virus mutants might replicate poorly in neuronal cells, thus accounting for their avirulence.

It was not until recently that the critical role of tk was understood. The function of the enzyme is to synthesize essential building blocks of DNA. If this synthesis is prevented, viruses cannot replicate and consequently cannot cause infection. Most tissues of the host have sufficient amounts of tk to support viral replication. Nondividing tissues such as nerve tissue lack tk and can only support viral DNA synthesis if the tk is provided by the infecting virus. The role of tk expression of PRV infection has been studied [18,20].

A number of investigators have shown that the virulence of herpesviruses is multifactorial [19,20,21]. However, one of the factors of primary importance for pathogenicity is thymidine kinase [22]. The degree of virulence was shown to be dependent on the amount of viral tk produced by the tk negative mutants.

It would appear that herpesviruses with deletion of all activity of the tk gene are desirable for vaccines, since the deletions of substantial size cannot revert to virulence [18,23]. The vaccines produced will be safe and should maintain their immunogenicity and reduce colonization of nervous tissues by virulent PRV strains. The effective control of PR requires the use of a vaccine which most often reduces the number of animals that become carriers. No vaccine can completely

Table 3.

New Strategies For Producing Vaccines

Nonreplicating Antigens
- Expression of protective antigens in
  —prokaryotic host cells—BTV, TGE, ROTA
  —eukaryotic host cells—HB_sAG
  [biological activity generally low]
- Synthetic peptide vaccines—FMDV
- Antiidiotype antibody vaccines—Rabies

Replicating Antigens
- Mutations—PRV
- Heterologous viruses—Rotoviruses
- Genetic reassortment—BTV influenza
  (applicable to viruses with segmented genomes)
- Gene Deletions/Additions—IBR, PRV, Vibrio cholera
- Living subunit vaccines
  —use of attenuated viruses as vectors for carrying foreign genes—PRV/HC
  —use of bacterial vectors for carrying foreign genes—Salmonella/toxin A

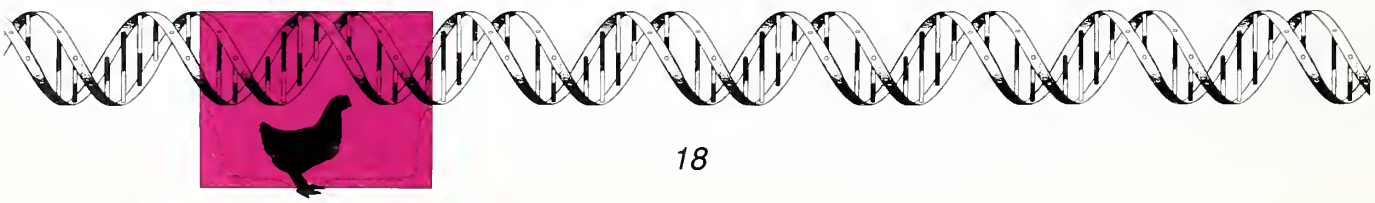


Figure 1. Conceptual Representation of Gene Deletion/Addition.

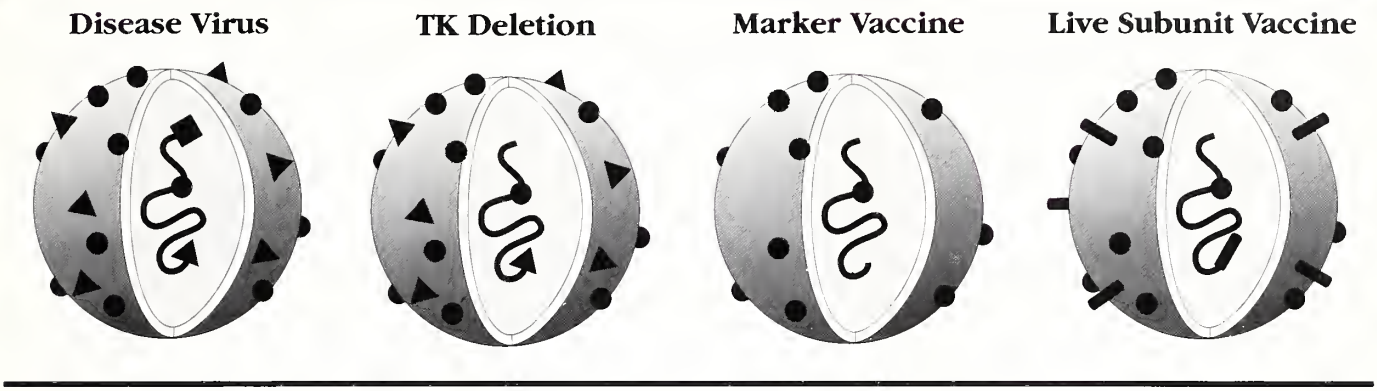


Table 4.

Nonreplicating Pseudorabies Vaccines

Vaccine	Company	Type	Diagnostic Marker
Porci-Rab	Beecham	Whole Organism	No
PR-Vac	Norden	Whole Organism	No
Suvaxyn-PRV	Solvey	Whole Organism	No
Geskypur	Rhone-Merieux	Sub-unit	Unknown
Breakstop	Molecular Genetics	Sub-unit	Yes not released

Table 5.

Replicating Pseudorabies Vaccines

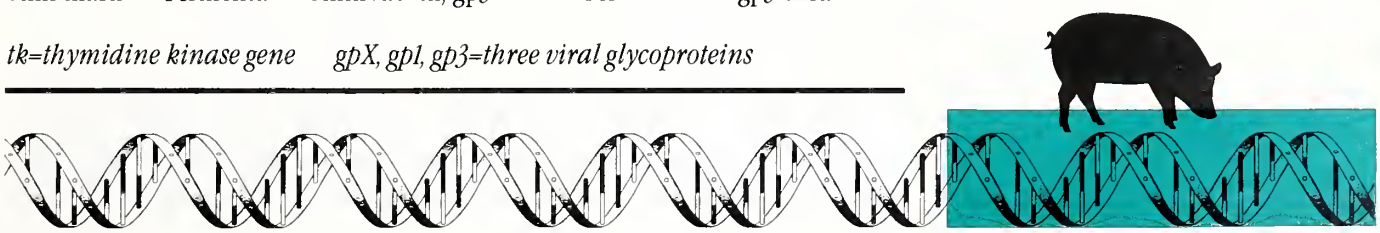
Vaccine	Company	Type	Gene Deletion	Diagnostic Marker	Comments
Pseudorabies	Bio-Ceutic	Bartha	gp1, gp63	Possible	Elisa
PR-VAC	Norden	Buk	gp1	Pending	Elisa
Omnivac-PRV	Fermenta	Buk	tk	None	
PRV-Marker	Syntrovet	Iowa	tk, gpX	Yes	gx Elisa
Tolvid	Upjohn	Auj.	tk, gpX	Yes	gpx Elisa
Omnimark	Fermenta	Omnivac	tk, gp3	Yes	gp3 Elisa

tk=thymidine kinase gene gpX, gp1, gp3=three viral glycoproteins

protect against animals becoming carriers.

The concept of gene deletions and their consequences are shown in figure 1. In the figure, PRV is shown in cutaway. The glycoprotein genes responsible for the antibody responses are on the envelope surface. Their corresponding gene loci are on the DNA. The tk deletion (snake head) removes virulence, leaving an intact virion suitable for use as a vaccine. Removal of an additional gene which codes for one of the glycoproteins now provides us with a "marker" vaccine. Lastly, if we insert an immunizing gene from an external source, we create a live subunit vaccine (also referred to as a vector vaccine).

Pseudorabies vaccines currently available for use or under development are shown in table 4 and table 5.



The genetic engineering of PRV is illustrated in figure 2. Line D shows the genome of PRV. Using restriction endonuclease analysis (DNA fingerprinting), molecular epidemiologists can identify and determine country or state of origin of field isolates. To date, 67 isolates of PRV have been tested and identified by Pirtle and associates at the National Animal Disease Center, Ames, Iowa [24]. The specific areas of interest include a) location of gp III, tk, gpX, gp I, b) the unique short region—Us—, and c) the terminal repeat region. The genomes of each of the genetically "engineered" vaccines are shown and compared with a wild isolate and with conventionally produced PR-VAC (Norden).

The OMNIMARK-PRV genome (F) illustrates the site of deleted genes GIII and tk plus the site of a mutation present in the parent Bucharest (BUK) vaccine strain. OMNIVAC-PRV is identical with the exception that GIII is not deleted.

The Norden vaccine (N) also derived from the BUK strain shows a deletion in the GI gene and a similar mutation in the Us region to that of OMNIVAC-PRV.

TOLVID vaccine produced by Upjohn (U) is derived from the original Aujeszky's isolate, and shows a deletion in the tk gene, plus a deletion of the GX gene from the Us region.

MARKER/PRV developed by Syntrovet (S) originated from the Iowa virulent strain. Again, deletions are tk, and GX, with an insertion of the beta galactosidase gene.

Figure 2. Schematic Representative of PRV Genome

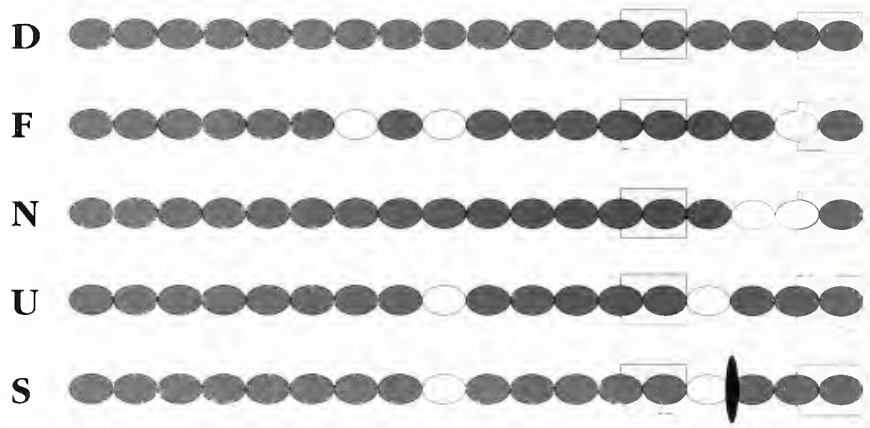


Table 6.

Comparisons of Gene Deleted PRV Vaccines

Parental Strain ▶ Properties ▼	**Syntro Iowa** (virulent)	**Upjohn Aujeszky** (virulent)	**Intervet NIA-3** (virulent)	**Fermenta Bucharest** (vaccine)
Genetically engineered to prevent reversion to virulence	Yes	Yes	No	Yes
Foreign gene inserts	Yes	No	No	No
Attenuation multigenic	Yes	No	Yes	Yes
Marker	GX	GX	GI	GIII
Potential for spread of marker negative recombinants derived from field strain and vaccine recombination	Yes	Yes	Yes	No

The tk deletion in PRV vaccines attenuates the virus, and prevents the vaccine from reproducing in the brain. Limited replication of the vaccine occurs in peripheral tissues and the vaccine elicits a complete cellular and humoral response. The tk negative strains of PRV are thought to reduce the extent to which superinfecting virulent virus can establish infections in neural tissues.

Further comparisons of these vaccines are shown in table 6. Notice that 3 of the 4 were derived from virulent virus strains, and 3 of the 4 have deleted antigens from the Unique Short region for their marker characteristic. Differences of significance center on a) safety in other species, and in newborn pigs, and b) in parental origin of the vaccine strain.

Marker Tests

The new vaccines appear to be more advantageous than currently available conventional vaccines, as they provide a greater degree of safety, especially for commingled domestic animals. Gene deletion also provides for the development of "marker" vaccines [25-29].

Vaccinated animals can now be differentiated from naturally infected animals based on the absence of antibodies to targeted antigens in sera of vaccinates. Three antigens from PRV have been deleted either by genetic engineering (gpIII, gpX) or by passage through aberrant hosts (GpI). The three PRV glycoproteins are being used as diagnostic test antigens in ELISA tests.

HERDCHECK is a PRV gpX antibody ELISA test. It is monoclonal antibody based and is potentially useful for both TOLVID and PRV/MARKER vaccines. The test kit was licensed by the USDA in 1988 and is now available at diagnostic laboratories on approval of State veterinarians.

A number of ELISA diagnostic kits are under development or in use for gpI vaccines. How effective each of the tests will be is yet to be determined. Two gpI test kits were recently compared by VanOirschot and Oei [30]. ELISA I, manufactured by Intervet, Oxmeir, The Netherlands, and ELISA II, developed by Central Veterinary Institute, Lelystad , The Netherlands, were equally sensitive in detecting antibodies to gpI in susceptible seronegative pigs. The ELISA I test, however, scored false positive results with sera from pigs repeatedly vaccinated with a gpI-negative vaccine. ELISA I differs from ELISA II in that the latter is based on the use of 2 monoclonals recognizing different epitopes on the same antigen. No published reports on the gpI test kit under development in the U.S. were found.

A new blocking ELISA to distinguish PRV-infected pigs from OMNIMARK-PRV-vaccinated pigs has been developed [29]. The PRV gpIII glycoprotein used in the test is considered a major immunogen [31,32]. The other markers, gpX and gpI, are minor glycoproteins. How much advantage this offers has yet to be determined. One published advantage to the gpIII test is the use of *undiluted* test serum samples. This is a time and materials saving feature for diagnostic laboratories. Application for licensing of the test kit has been submitted to the USDA.

Conclusion

This presentation omits the evolution of vaccines and how effective vaccines have been in disease control. The transition to new vaccines is also incompletely documented. Four presentations made recently are recommended for additional reading:

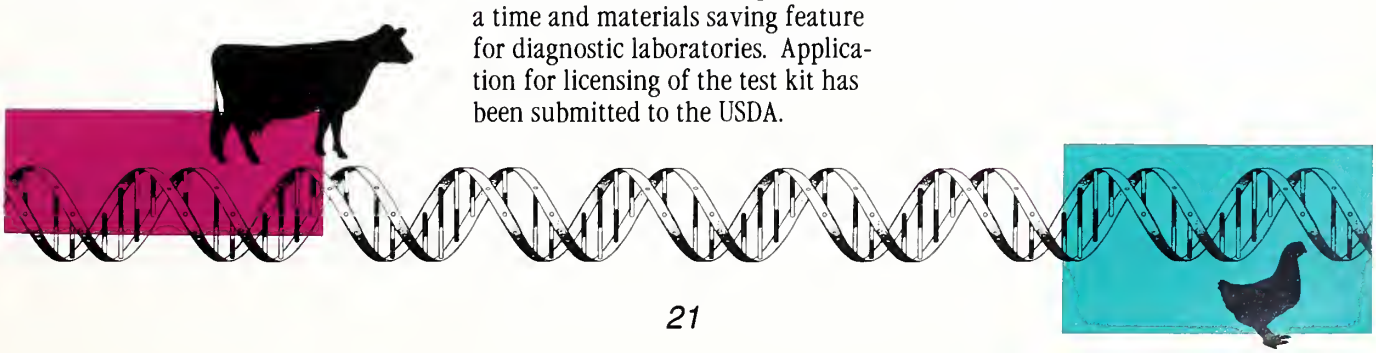
• Kit, S. (1986) Genetic Engineering of Novel Animal Vaccines. Proc 90th Ann Mtg USAHA, Louisville, KY.

• Molitor, T., Thawley, D. (1987) Pseudorabies Vaccines: Past, Present and Future. Compendium Food Animal: Swine Continuing Education Article, vol 9: F409-F416.

• Wedman E.E. (1988). Designing genes to make vaccines—Imagine the possibilities. Am Assn Swine Pract Ann Mtg pp 69-74.

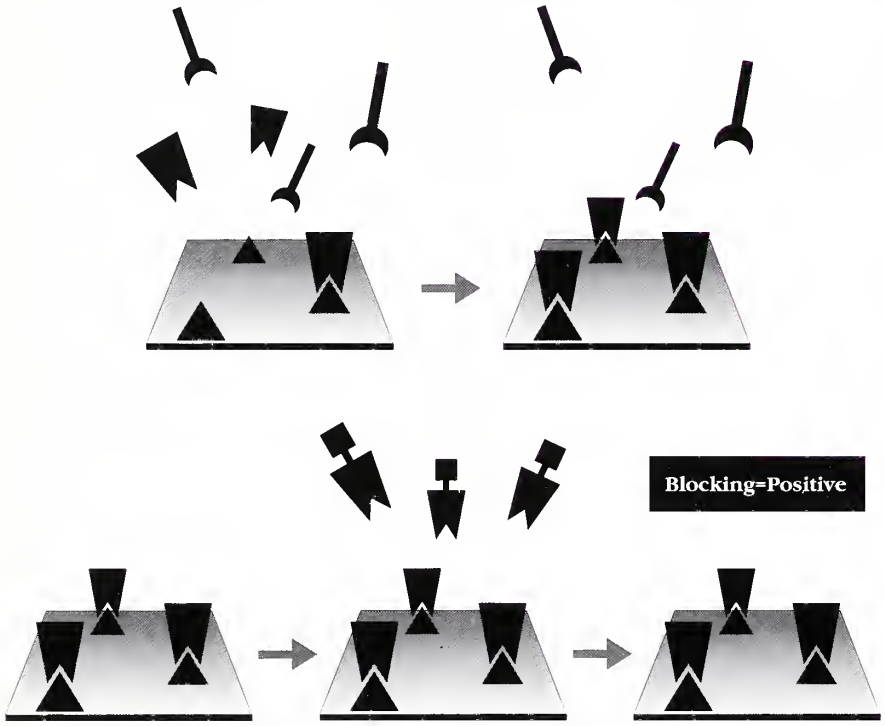
• Babiuk, L.A. (1988). Advances in Viral Vaccine Technology. 21st Ann Conv Assn Bov Pract. Alberta, Canada.

What does biotechnology have to offer vaccine development? Recent developments will revolutionize the practice of our profession. There will be an improvement in current vaccines as well as the development of vaccines that are not now available. Marker vaccines will become the standard. As these developments progress, we will be more able to manage illness and eliminate disease. It is now up to each of us to keep abreast of these new technologies and be ready to use them for the benefit of our clients.



Vaccinated Pigs Serological Response to Marker Vaccine.

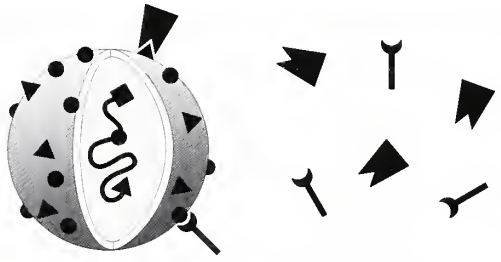
Blocker Positive



Legend: Differential ELISA

- Test sera added to wells of microtiter plate. Antibodies to marker antigen absorb to marker antigen bound to wells.
- Test sera removed by washing, only absorbed antibodies remain.
- Conjugate added: Peroxidase labeled anti-marker antigen monoclonal, allowed to absorb, then washed off if unbound.
- Sera of Pseudorabies Disease exposed animal: sera has Blocking antibodies, no conjugate is bound; Positive test.
  Sera of Marker Vaccinated animal: sera lacks blocking antibodies, conjugate is bound; negative test.

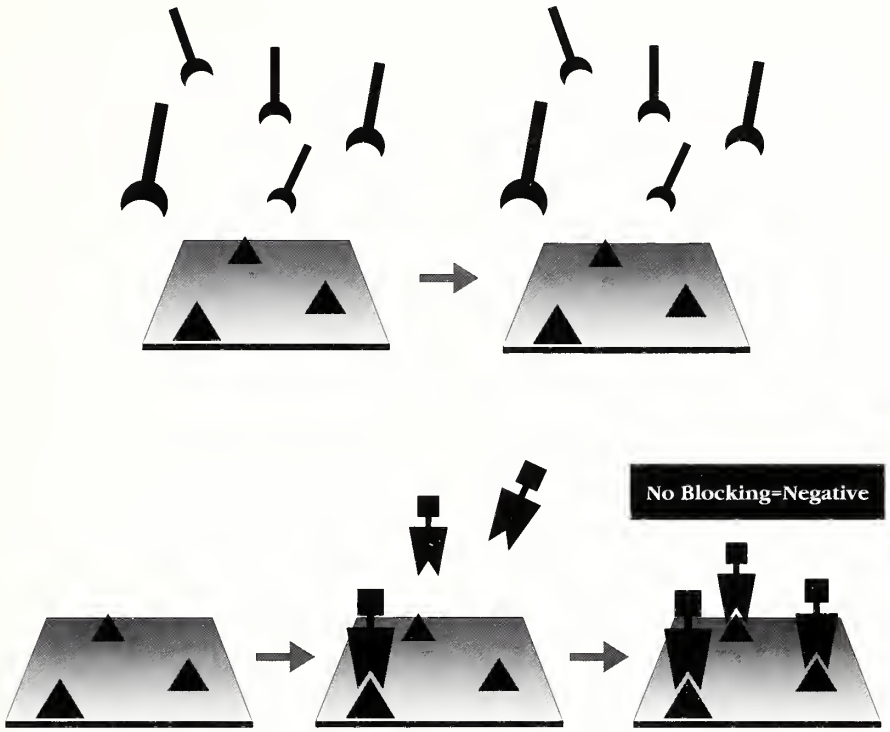
Total Serological Response to a Natural Infection.



Pigs Make Antibodies to Disease Virus

Pigs Make Antibodies to Marker Vaccine

Blocker Negative



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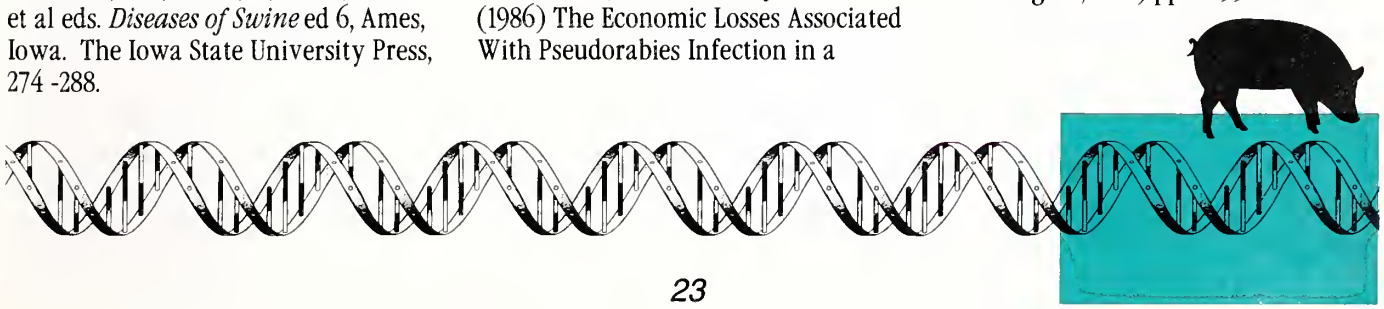

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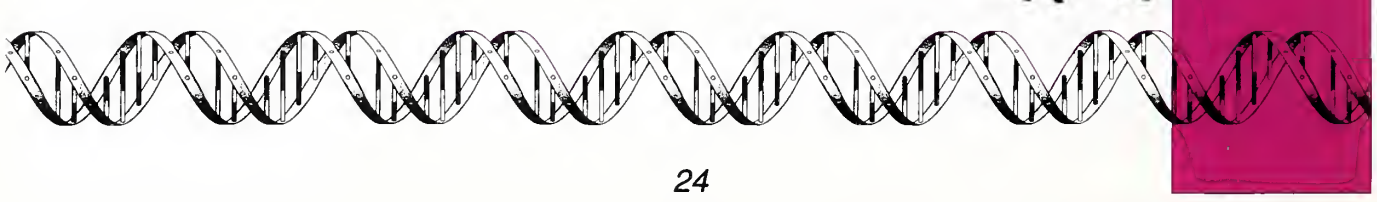

Vaccinia Vectored Vaccines

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During the last century of advances in combined veterinary virology-vaccinology, a gradual technological metamorphosis has occurred. The ancestral first generation viral vaccines which had long dominated the field, often little more than purified organ homogenates of infectious material, have slowly yielded to a second generation of cell culture-derived products, albeit often with unrealistic price tags, especially to developing national needs. Recent technical advances in cloning and gene expression have led to the evolution of a third generation of products, recombinant virus vaccines, partially offering a solution to this dilemma. One such expression vector candidate, the Orthopoxvirus vaccinia, has been extensively studied and well characterized. Its large genome size, ease of production, thermostability, broad host range, and impressive safety record acquired during the smallpox eradication campaign combine to make vaccinia-vectored viruses ideal for the expression of foreign genes in animal models. Earlier, workers demonstrated the utility of vaccinia virus constructs which could encode for a variety of antigens (e.g., hepatitis, herpes, influenza viruses, etc.). The cognate expression of foreign proteins by a self-replicating system offered the potential for long-term animal immunization without the overt risk of disease from the inciting etiological agents of interest. The likely benefits and accompanying controversies associated with vaccinia recombinant virus research may be best illustrated by the example provided by the development of a vaccine designed to control rabies in wildlife.

The seemingly novel concept of wildlife vaccination against rabies by the oral route is certainly not an entirely new idea; it was envisioned in the laboratory by the "Father of Oral Rabies Immunization," Dr. George Baer, nearly three decades ago. Thereafter, the initial field experiments by Swiss and German researchers demonstrated, by means of a bait-containing vaccine, that free-ranging red foxes could be immunized against rabies safely, economically, and effectively more than 10 years ago. Recently in North America, Canadian investigators have used synthetic vaccine-laden baits containing attenuated rabies virus in attempts at control of endemic fox rabies in southern Ontario. Unfortunately, as individual species' susceptibilities differ greatly depending upon the strain of rabies virus, mammalian responses to oral rabies vaccine also vary dramatically. Red foxes are sensitive to dosages of attenuated or modified-life virus (MLV) vaccines that do not effectively immunize important rabies reservoirs such as dogs, skunks, or raccoons. For example, it requires approximately a 100-fold increase in MLV rabies vaccine to orally immunize raccoons as opposed to red foxes. This may represent a significant factor in vaccine production costs. An added disadvantage of traditional MLV vaccines is the potential for iatrogenic disease in the host intended for immunization. Such actual and theoretical drawbacks to oral vaccination, coupled with considerable ignorance of the applied ecology of many species, created a historical impasse to rabies elimination.

In contrast to aspects of its intricate epizootiology, confounding effective control attempts historically, rabies virus is antigenically less complex. Rabies is caused by a rod-shaped, single-stranded, negative-sense, non-segmented RNA lyssavirus, composed of five structural proteins. Three of these are complexed to the viral genome to form an inner helical ribonucleoprotein complex, or nucleocapsid, surrounded by glycoprotein spikes anchored to an outer lipid membrane. These viral glycoprotein surface projections induce the formation of rabies-specific virus neutralizing antibodies (VNA), which are considered to be important in viral immuno-prophylaxis. Advances in the study of the molecular biology of rabies viruses, concomitant with development of vaccinia recombinant virus technology, led to the construction of a vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine. This was accomplished by the insertion of a plasmid bearing a cDNA copy of the ERA strain rabies virus glycoprotein gene within the thymidine kinase (TK) locus of the so-called "Copenhagen" strain of vaccinia. When grown in cell culture, the V-RG virus produced large amounts of rabies glycoprotein that could not be differentiated antigenically from authentic

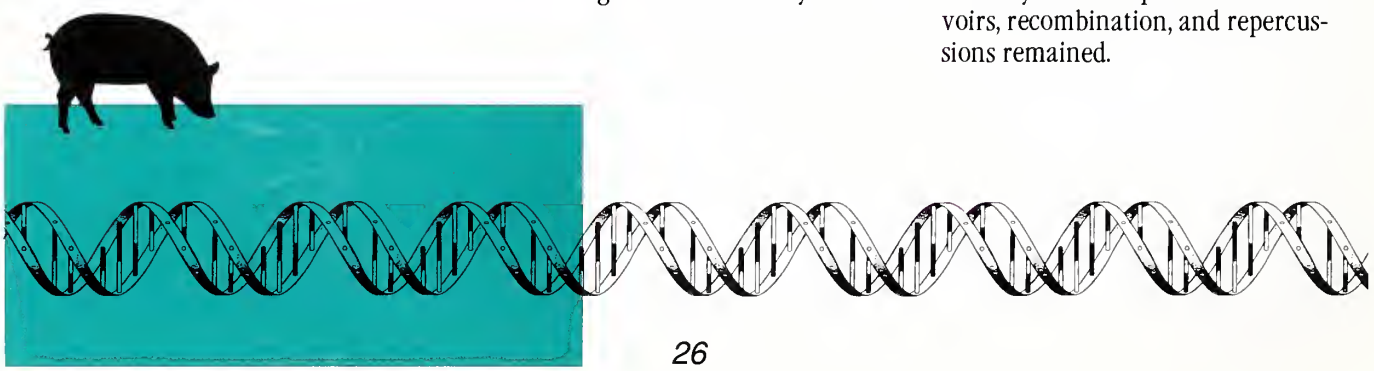
viral glycoprotein. Moreover, when inoculated parenterally into laboratory animals, the V-RG virus led to the rapid formation of high levels of rabies VNA and successful protection against several antigenically distinct street rabies viruses, indicating its potential as a human or veterinary vaccine.

The resulting V-RG recombinant virus shares many properties with its parental vaccinia virus but differs in other ways, all of which make the V-RG recombinant virus an ideal vaccine for animals. Among the differing properties, one of the most important is the attenuation (reduced virulence) of the V-RG recombinant virus in comparison with its parental virus. Inactivation of the TK gene by insertion of another gene (in this case the rabies glycoprotein gene) in the recombinant vaccinia virus dramatically decreases, by several orders of magnitude, the virulence of mice by the i.c. and i.p. routes. Insertional inactivation of the vaccinia virus TK gene in the recombinant virus can be compared with the parental virus by measuring the diameter of the test lesion at the site of inoculation and the dose of virus required to ulcerate the skin. The diameter of the lesion is smaller and the dose required to create a lesion is larger with the V-RG recombinant virus. In a comparative study with the V-RG recombinant virus and parental Copenhagen strain, inoculated footpad and i.p. into immunodepressed 6-week-old female Swiss nude/nude mice, only the parental virus caused lesions at the higher dose when administered by the i.p. route (no lesions were detected with the V-RG recombinant virus administered i.p. or in the footpad).

Comparatively, both viruses replicate normally in BHK-21 and Vero cells (two cell types used for large batch virus production). Both viruses form similar small-size round plaques in cell culture. Comet plaques characteristic of virulent vaccinia virus strains are not formed by either virus. The BHK-21 cell monolayers infected with the V-RG recombinant virus reveal that the glycoprotein expressed from the rabies glycoprotein gene sequence is present in the cytoplasm of acetone-fixed cells and at the cell surface, suggesting that the recombinant replicates in the cytoplasm of infected cells as does the parental virus.

The V-RG recombinant virus was not originally developed as an oral wildlife rabies vaccine, *per se*. It was originally conceived as an expensive alternative veterinary vaccine to be used by the parenteral route of administration. However, several events in combination shaped subsequent events. The intensity of the mid-Atlantic raccoon rabies epizootic, coupled with the inadequacy of existing vaccines to immunize raccoons orally, together with the observation that V-RG could induce rabies VNA when given to laboratory rodents *per se*, led to a series of experiments which demonstrated that raccoons and a variety of other carnivores could auto-vaccinate via the consumption of vaccine-laden baits.

Understandably, early research concerns focused upon vaccine safety. Over the past 6 years, the V-RG vaccine has been tested in captivity in more than six avian and 35 different mammalian species, including the majority of global rabies reservoir hosts and significant non-target species. Regardless of vaccine dose or route, animals have remained clinically normal, with no overt gross or histo-pathological lesions. The V-RG vaccine is cleared relatively quickly (e.g., 48 hrs) after oral deposition. The tonsils and local lymph nodes appear to be key sites for early viral reception. The acidity and enzymatic milieu of the stomach and post-gastric intestinal tract inactivate residual virus. True biological transmission has not been observed, although mechanical transmission is a predictable and documented event (e.g., a vaccinated lactating female raccoon grooming her suckling kits). Passive transmission of immunity may occur if vaccination is anticipated during pregnancy. The abortifacient, teratogenic, or oncogenic potential of V-RG has not been realized. Considering the diversity of animal species tested, the dosage concentrations, and the variety of routes employed, the V-RG vaccine has become one of the most extensively tested rabies biologicals in recent memory. Further questions of reservoirs, recombination, and repercussions remained.



Would the experiemental field use of the V-RG vaccine for wildlife rabies control result in the establishment of vaccinia animal reservoirs in nature? Although no longer in routine clinical use for the last decade, vaccinia virus was used as a human vaccine for almost 200 years since its introduction by Edward Jenner for immunoprophylaxis against smallpox. During the worldwide smallpox eradication campaign, vaccinia virus was used in the millions of doses by intentional scarification with high concentrations of dermotropic-adapted virus and often under less than ideal circumstances, especially in the humid tropical regions of the globe. Unquestionably within these niches, the ecological opportunity for passage and establishment of virus from humans to domestic animals frequently existed, and contact transmission was demonstrable (e.g., bovine milkers). Considering the intensity of the human-animal bond within certain societies, these instances were more infrequent than otherwise predicted, usually self-limiting, and without perpetuation. Moreover, despite exhaustive speculation, the evolutionary derivation (i.e., from variola, cowpox, or horsepox by hybridization or adaptation) and original natural hosts of vaccinia virus are still unclear and will probably remain unanswered. Thus, vaccinia virus appears best regarded as a laboratory virus (nonexistent in nature) for which humans were the intentional principal host. Although vaccinia virus has a wide animal host range (in experimental infection), there is no good evidence that vaccinia virus has become established in animal populations by natural infection. Anticipated field trials with the V-RG virus are not expected to alter this longstanding situation.

Obviously no self-replicating system, either MLV or recombinant-based vaccine, is totally risk-free. Unarguably, vaccinia virus is a minor human pathogen. When inoculated into superficial layers of skin, it produces a lesion characterized by hyperplasia, viral proliferation and inflammatory infiltration that generally progresses from a papule through a vesicle and pustule to a crust, followed by healing with a scar. A transient viremia is possible, but generalized lesions are extremely rare in the immunocompetent host. The occasional abnormal reactions range from mild, local ulceration to as severe as vaccine-induced mortality, broadly classified as dermal in origin or involving the CNS (e.g., postinfectious encephalitis, with signals referable to a generalized viral encephalitis). Rates for post-vaccinal encephalitis (all ages) in the U.S. averaged 3 per million primary vaccinia virus vaccinees.

Attention to the parental vaccinia virus origin, passage history, and characteristics of the new recombinant viral species are especially important, because during the smallpox eradication campaign it was widely believed that the several vaccinia virus vaccine strains used in different countries greatly contributed to the variation of human complication rates observed, particularly post-vaccinal encephalitis. Although vaccinia virus strains may differ in their virulence in regards to the inoculation of suckling mice, direct correlation of these experimental results to clinical complications in vaccinated humans, such as post-vaccinal encephalitis, was poor. Additionally, the exact etiology for many of the reactions (e.g., human post-vaccinal encephalitis) was oftentimes not determined. Vaccinia virus was not routinely isolated from the CNS lesions; the nature of the complication was thought to be allergenic, with no known predilections. Mortality usually ranged from 10-30%. The considerable variation in incidence of CNS complications was never adequately explained. Furthermore, robust experimental animal models were never developed to adequately mimic postulated cause-effect relationships of so-called more virulent vaccinia virus strains and associated human complications, and no firm correlations were established from the results of neurovirulence testing of different vaccinia virus strains in animals. Epidemiological indications that the primary difference in complication rates observed between countries was primarily due to the specific vaccinia virus strains used, were also poor.

Complication frequencies in different countries, and even in the same country, varied tremendously from year to year. During 1956-70, Denmark reported 20 cases of post-vaccinal encephalitis per million primary

vaccinees using the Copenhagen strain, compared to 9 per million in the United Kingdom during 1961-70, and 3 per million in the U.S. for 1968. Yet no significantly higher rates of mortality from post-vaccinal encephalitis using the Copenhagen strain were reported: 3.3 per million for Denmark, compared to 2.5 per million for Sweden and 2.2 per million for Great Britian and Wales, where the Copenhagen strain was not used. Moreover, while almost a two-fold decrease in post-vaccinal encephalitis was reported for the Netherlands after a 1962 switch from using the Copenhagen strain (used during 1959-62) to the Elstree strain (used during 1963-66), a further five-fold reduction was recorded for the period 1967-70, when only the Elstree strain was used. Thus, other factors, such as adherence to accepted contra-indications to vaccinia vaccination (e.g., age, eczema, etc.), inadvertent inclusion of allergic proteins in the vaccine, bacterial contamination, etc., may have played a major role in the lowering of complication rates. Finally, the relatively low rate of serious post-vaccinal complications overall, the lack of consistency in case definitions required for diagnostic reporting following vaccinia immunization, and the frequent omission of denominators for number of primary vaccinees and the population ages compared means that a direct comparison of different vaccinia virus strains from retrospective epidemiological data, at least as regards potential human effects, is an impracticality and nearly incalculable when considering the more attenuated recombinant vaccinia-vectored vaccines for which humans are clearly not the primary targets.

All know CNS and dermal complications of vaccinia in humans were associated with the act of intentional intradermal inoculation of high concentrations of parental vaccinia virus. Considering the attenuated nature of the V-RG recombinant virus (even before TK gene inactivation, by the adaptation of its parental strain to egg and cell culture) and the proposed restricted nature of its use, it is extremely unlikely that such vaccine-induced complications would occur in humans from the field application of vaccine-laden bait intended for wildlife, especially because the risks associated with primary vaccination were greatest during the first years of life.

What are the theoretical concerns over the potential reproduction/capacity for genetic transfer between recombinant viruses? Orthopoxviruses belong to a single genus within the sub-family of pox viruses of vertebrates. The principle members are vaccinia, various, cowpox, monkeypox, camelpox, raccoon pox (RP) virus, volepox, taterapox (gerbil), and Uasin gishu (isolated from a horse in Kenya), and mousepox (ectromelia). The RP virus is the only New World orthopoxvirus found in the Eastern United States. Vaccinia virus, which replicates in the cytoplasm of infected cells, relies on virion-associated enzymes for viral DNA and progeny virus reproduction. The DNA sequences of the total gene pool of vaccinia virus are arranged fairly consistently with other orthopoxvirion genomes; the functional genes are in a highly conserved central part of the linear DNA genome, whereas the arrangements of sequences in the other sections of the genome tend to be characteristic of the different species of orthopoxviruses. Mapping studies have detected genetic mutations and deletions near both termini of the genome. Variants of parent virus occasionally contain inverted terminal repeats that are larger than those in the parent virus and sometimes involve duplication of additional sequences mapping at the opposite end of the parental genome. It is unclear whether such structures could be derived by a single recombination event between two worldtype genomes aligned in opposite polarity or in a more complex manner, involving mutant DNA's (or different virus genomes) simultaneously replicating a single host cell in vivo. Compared with the high frequency of intramolecular recombination that occurs in vitro under nonselective conditions in cell culture, the probability of recombination occurring naturally is extremely low, as illustrated.

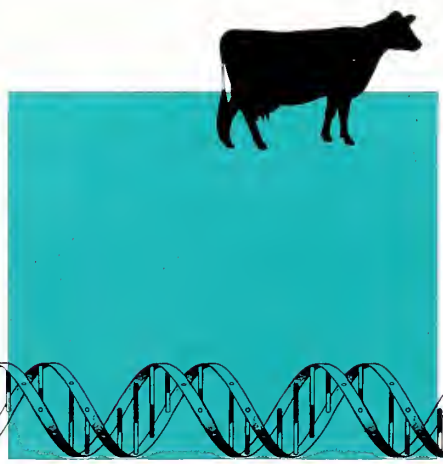
In order for orthopoxvirus recombination to occur, simultaneous infection with two viruses is necessary. The probability for such a recombination event between V-RG recombinant virus and another orthopoxvirus depends upon the relative point prevalence of each. A theoretical intended herd immunity level of 70% with V-RG recombinant vaccine contact in raccoons resulting from vaccination would be ideal; since virus

is cleared readily from orally-inoculated animals (within 48 hrs), either recombination between the vaccine virus and an indigenous virus must occur within this very restricted period of time or it is necessary to postulate a latency factor or persistent infection, although none has been found among nearly a hundred raccoons tested with V-RG recombinant virus in captivity thus far. As such, the probability in which animals are persistently infected with the V-RG recombinant virus equates to a putative order of magnitude less than 1%. A similar probability for persistent infection of less than 1% must also be postulated for RP virusinfection in racoons. Although previous surveys suggested a seroprevalence of RP virus as high as 20%, there is no substantial evidence of RP virus infection among raccoons on the basis of several hundred racoon sera screened for anti-RP VNA activity. Moreover, there have been only two isolations of RP virus, more than 20 years ago at a single geographical location, with no further reports throughout the U.S., suggesting a prevalence less than 0.1% at present. In this regard, the probability product of simultaneous or super infection with V-RG recombinant virus and RP virus in the same animal, and genome contact in the same cell with regeneration of an intact TK gene, is on an order of magnitude of 10^{-8} or less.

Thus, the probability of recombination of the V-RG recombinant virus with a naturally occurring orthopoxvirus and consequent regeneration of a vaccinia virus (possessing an intact TK gene) is very low. The chance of recombination between V-RG recombinant virus and RP virus, resulting in regeneration of vaccinia virus lacking the rabies virus glycoprotein gene but possessing an active (V-RG) recombinant virus/RP TK gene, occurring in the natural environment, depends upon the natural prevalence of RP and induced herd immunity with V-RG recombinant virus, as detailed above. As far as recombination of the V-RG recombinant virus with various indigenous avipoxviruses, or other orthopoxivruses, the probability will vary with the taxonomic relationship of the indigenous pox virus to the V-RG recombinant virus. If a relatively unrelated wild virus (e.g., a leporipoxvirus) is involved, then the probability of inheriting a second TK gene or reconstructing the original TK gene of the vaccinia vector is no more likely than the exchange of any other gene between these two viruses. Conceivably, illegitimate recombination with a totally unrelated DNA virus could occur, but it would be extremely unlikely.

Objectives of current research are to conduct a three-phase program to field-test an oral rabies vaccine for use in raccoons and other carnivores. Phase I entails the investigation of raccoon ecology and the distribution and testing of biomarker and placebo bait delivery systems on several potential study sites; Phase II involves the actual field testing of the oral V-RG recombinant vaccine incorporated in the bait system to determine its safety and immunogenicity under field conditions for target and non-target species; Phase III involves the laboratory challenge of selected individual wild-caught vaccinated raccoons from the study population with rabies street virus to assess limited efficacy (protection). After these preliminary steps have been taken, controlled field studies will attempt elimination at natural epizootic foci; similar work has progressed beyond these preliminary steps in Belgium and France, where the V-RG vaccine is being evaluated for fox rabies control.

Over the next decade, recombinant vaccines will be used in the global control of many infectious human, domestic animal and wildlife diseases with increasing frequency. The advantages of greater safety, improved efficacy, overt versatility and proven reliability will gradually supplant less effective first and second-generation viral vaccines of the past. Although vaccinia-based vaccines were among the first to be widely exploited in the laboratory, other recombinant viral vectors, such as adeno-, baculo- and herpes viruses, will also contribute to disease control as various epidemiological demands necessitate. Socioeconomic analyses will demonstrate that the relative risks of not using such genetically engineered vaccines (and hence supporting the infectious disease



status quo) far outweigh the perceived environmental or public health hazards associated with biotechnology. It is the collective responsibility of biomedical researchers and practitioners alike to comprehend public concerns, differentiate science fact from science fiction, and allay the misconceptions and fears that the pending "brave new world" will surely produce.

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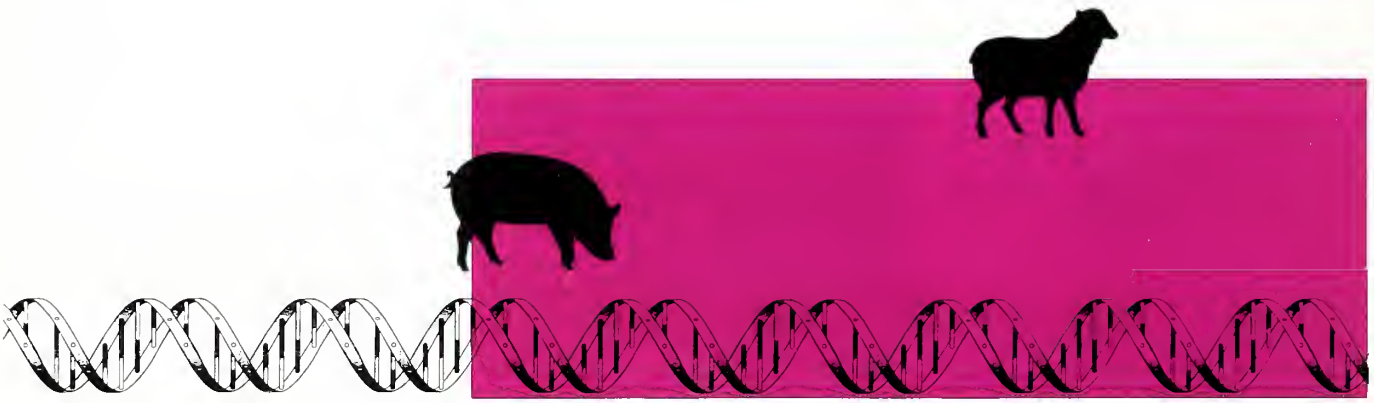

Transgenic Animals

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Animals carrying and expressing foreign genes represent one of the most elegant and potentially useful applications of modern biotechnology to animal science and production, and may have a significant impact on veterinary medicine. An animal is considered "transgenic" provided the foreign gene is carried in the germ line. The inserted gene, which may be derived from another individual of the same species or another species, is termed a "transgene." To ensure proper expression the transgene is usually linked with a promoter sequence (gene), which also may be of foreign origin.

Why Make a Transgenic Animal?
For centuries man has manipulated the genetic composition of agricultural and companion animals through selective breeding programs. Traditional goals for food animals are to increase production of meat, milk, and eggs; change fat-to-lean ratios; increase reproductive efficiency; and increase disease resistance. Breeding programs for companion animals have emphasized conformation, physical fitness, disease resistance, and disposition.

Disease resistance, directed either to the causative organism or to increased responsiveness to vaccines, is of special interest to veterinarians. Genes that influence disease resistance have been linked to the major histocompatibility complex (MHC) or to the production of lymphokines and interferon, specific immunoglobulin molecules, and molecules which block cellular receptors for or replication of specific pathogens. The molecular structure of some of these genes is now known.

Scientists expect that through transgenic technologies traditional breeding goals may be realized more quickly, since a single useful gene may be introduced to a population in the absence of other undesirable genes.

Other nontraditional goals include the treatment of disease through gene therapy, either by correction of a genetic defect or by supplying a therapeutic gene product. Also, transgenic animals represent a means for the production of large quantities of uniquely valuable proteins.

Although some conjure images of monsters and new species, experience to date supports the expectation of scientists that phenotypic changes will be very subtle and can be controlled.

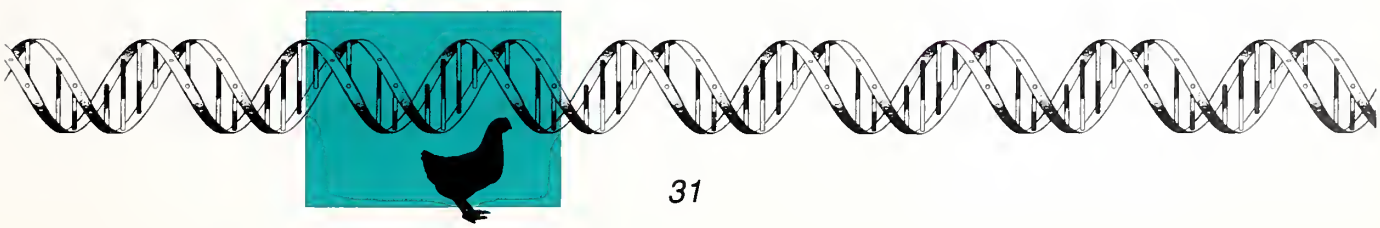
How Do We Make a Transgenic Animal?
The techniques used to produce transgenic animals vary but all involve the introduction of the target gene, usually coupled to a promoter, to the fertilized ovum or developing embryo at a very early stage. Microinjection has been the method of choice for most mammalian species and fish. The test material is introduced directly into the nucleus of the ovum shortly after fertilization.

In chickens, genes have been inserted via infection with retroviral vectors since the physical nature of the ovum precludes microinjection. Retroviruses integrate into the host-cell DNA during their replicative cycle. They also can carry a small amount of extra genetic material. A variety of vector constructs have been studied that vary in their oncogenicity and efficiency of replication.

Other methods, such as the transfer to embryos of cultured embryonic stem cells into which foreign DNA has been inserted by microinoculation or electroporation, show promise but are not yet in wide use.

Examples of Progress in Animals
Since the initial insertion of foreign genes in mice, a number of species important to veterinary medicine have been made transgenic. These include the chicken, cow, fish, pig, rabbit, mouse, rat, and sheep. The inserted genes for these pioneering efforts have consisted mainly of retroviral genes (used in chickens) and growth regulating genes of several species (used in mammals and fish). The experiences of two research groups have been selected to illustrate the extent of progress to date.

The pig story: A collaborative effort between scientists at the USDA-ARS Beltsville Agricultural Research Center and the School of Veterinary Medicine, University of Pennsylvania,[1] has yielded considerable progress in the production of transgenic swine carrying various growth-regulating genes. When fertilized pig ova were microinjected with the gene for bovine



growth hormone (bGH) coupled to a mouse metallothionein promoter, about 7 percent of the resulting progeny were transgenic. Of these, about two-thirds expressed bGH. When on an appropriate diet transgenic pigs grew about 11 percent faster and with a 17-percent improvement in feed efficiency. Also, subcutaneous back fat was reduced markedly from 21 mm to 7.5 mm. However, a number of adverse side effects were noted including depression of appetite and the production of lesions such as gastric ulcers, arthritis, cardiomegaly, dermatitis, and renal disease. These effects were similar to those induced by long-term inoculation of bGH. The authors suggest that strict regulation of transgene expression to a 4- to 8-week period during the rapid growth phase may obviate these side effects, but this desired result has not yet been achieved.

The chicken story: A collaborative effort between scientists at the USDA-ARS Regional Poultry Research Laboratory and the Frederick Cancer Research Facility [2,3] has resulted in the production of transgenic chickens carrying genes associated with avian leukosis virus, a common retrovirus of chickens. Developing chicken embryos were infected with live avian leukosis virus by inoculation into the yolk of unincubated, fertile eggs. About 24 percent of the male progeny were transgenic (but mosaic) and produced transgenic progeny at rates from 1 to 11 percent. The inserted genes were distributed to the third generation in Mendelian ratios. Twenty-three stable inserts were analyzed, 2 of which were defective and did not produce infectious virus. One of these, designated *alv6*, produced viral envelope glycoprotein which caused complete resistance in chickens to infection by subgroup A avian leukosis virus through the well-known mechanism of receptor blockage (interference). The possibility of using this transgene for control of avian leukosis, however, has been dampened by recent findings of oncogenicity in certain chicken lines. These findings may be explained by recombination of *alv6* and endogenous leukosis viruses to yield a virus with oncogenic potential (Crittenden, unpublished data).

Both examples illustrate not only amazing technical progress and promise, but also the need for further work before these model systems can be reduced to commercial practice.

Obstacles to Progress

We should not be surprised to find, at this early stage, that the transgenic road is full of curves and potholes that impede progress toward commercial implementation. There are many relevant issues, several of which are described as follows:

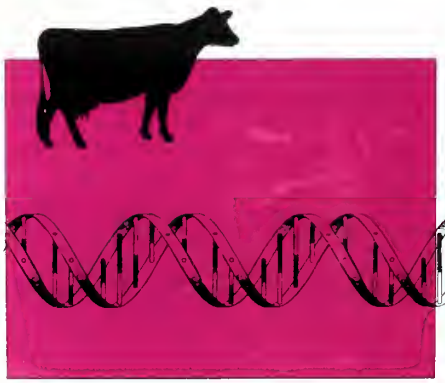
Lack of identified genes. A significant limitation is the relative lack of useful genes available for insertion. Genes influencing productive traits are largely unknown and uncloned. Efforts to map the genome of domestic animals, in a manner like the human genome project, may provide valuable information on gene function, but at considerable expense.

Insertion efficiency. The proportion of inoculated or infected ova that yield transgenic progeny is low. However, improved methods are under development and even the existing techniques could be considered for commercial use. This obstacle is not insurmountable.

Gene expression and regulation. Much needs to be learned of ways to optimize the expression of inserted genes and, even more importantly, to target the expression to occur in certain tissues or at specified times (ages). This field of science is being actively pursued and promising results are being obtained.

Multigenerational studies. A considerable amount of time will be required to prove that a gene has been stably inserted and to characterize its effects. A number of generations of traditional breeding will be mandatory and, as we have seen, the final product may not necessarily be useful.

Application to industrial breeding. Even after a gene has been inserted into animals with breeding potential, a substantial number of generations will be required for testing and evaluation, and then for introduction to the parent populations. This process is lengthy and complex, and is perhaps only slightly better than the introduction of a new gene by conventional breeding. The time required for this process adds significant costs.



Safety issues. Inserted genes obviously have the potential for disrupting normal function. Also, the vectors used for delivering the transgene may be detrimental (as in the case of retroviruses). Whereas adverse side effects need to be addressed during development of commercially useful animals, this need not be an overriding concern since many incentives exist to prevent the intentional or inadvertant commercial introduction of inferior animals.

Regulatory issues. Regulation of transgenic animals is provided by USDA-APHIS. Import, export, interstate movement, and field testing all require permits. The main concerns relate to the danger such animals present to other animal populations through disease or genetic contamination. Environmental concerns are significant for species that are difficult to confine, such as fish. The USDA-FSIS regulates animals that enter the human food chain. Guidelines for experiments with genetically-modified organisms including transgenic animals outside laboratory containment are being developed by USDA's Office of Agricultural Biotechnology.

Public opinion issues. The public has voiced its concern on the environmental release of transgenic organisms, including animals. Some question the ethical basis for "contaminating" the genome of an animal with genes from a foreign origin. Given the public concern about milk from animals inoculated with somatotropins, the importance of public image cannot be underestimated.

Patents. In 1988, the first patent on a transgenic mouse was granted to Harvard University. Although the patenting of plants and microoganisms is now accepted, many argue that animal life should not be proprietary. Some argue that the patenting of animals would adversely influence the economics of the small farm community. Legislation designed to restrict and regulate the patenting of animals is being actively pursued and the outcome of this issue is still not clear.

Impact on the Veterinarian and Veterinary Medicine

Commercial applications of transgenic animals may present new problems as well as new solutions for the veterinarian, but no major impact is expected in the short run. Animals in which growth rates are increased may be more prone to skeletal problems. Animals in which egg or milk production is increased or which have greater fecundity may have different nutritional requirements and be more susceptible to diseases exacerbated by stress. Reference has already been made to the possibility of pathogenic side effects. However, these problems are no different in type from those already experienced through conventional breeding. On the other hand, veterinarians will have new tools to control disease and may find ways to markedly reduce loss in both farm and companion animals due to specified diseases.

Conclusions

Transgenic science will ultimately create the technology to efficiently create animals with desirable characteristics. However, the introduction of such animals in food production or as companion animals may not be rapid. Probably the most immediate applications will be in the creation of animal models for human disease and animals which produce large quantities of useful gene products. Veterinarians will surely play a key role in health and safety issues and in providing information to elevate public knowledge and understanding. They will be uniquely qualified to provide input to those who regulate the health of livestock and the safety of food products. Above all, they will be guardians of the welfare of this important new class of animals as it inevitably gains public acceptance and enters into commercial use.

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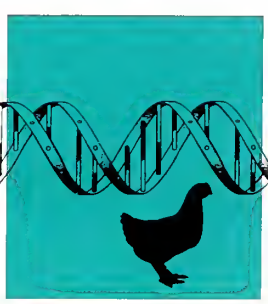

Bovine Somatotropin

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Bovine Somatotropin (BST) is an issue in which many different people are interested for many different reasons. BST is mentioned in the same breath with food safety, animal safety, the food supply, agricultural production and, particularly, biotechnology. Within all of these areas, the basic facts about BST remain the same. I want to review those key points and then briefly touch on efforts to inform consumers about BST by Monsanto and the other companies working on BST products.

First, for some background, I'd like to touch briefly on biotechnology because BST is a product of biotechnology. And while BST still is coming, biotechnology already is here. A number of lifesaving human pharmaceuticals produced through biotechnology are now in use...human insulin for diabetics, human growth hormone for children with growth deficiencies, interferon as well as other disease-fighting treatments, and the animal pharmaceutical products now being used to identify or cure diseases in cattle, hogs and other livestock.

We view biotechnology as the next logical step in the continued progression of technology that has made our human and animal health care and agricultural production systems the best in the world. Biotechnology employs natural processes less likely to pose risks to people or the environment. It allows us to improve plants in very precise and controllable ways. It also may bring significant environmental benefits in terms of less pesticide and fertilizer use and greater productivity from fewer animals.

Let us review the basic facts.

First, BST is a naturally occurring protein hormone made by cows for milk production. Biotechnology has made possible a manufactured version of this growth hormone. BST is a protein hormone, like insulin, not a sex hormone, like estrogen. This means that it does not accumulate in the body, but rather is quickly decomposed as any other food protein.

Second, milk from cows given extra BST is safe and is the same as any other milk. It has no significant differences in composition. So said the Food and Drug Administration—four years ago—and so say regulatory bodies in Western Europe and elsewhere in the world.

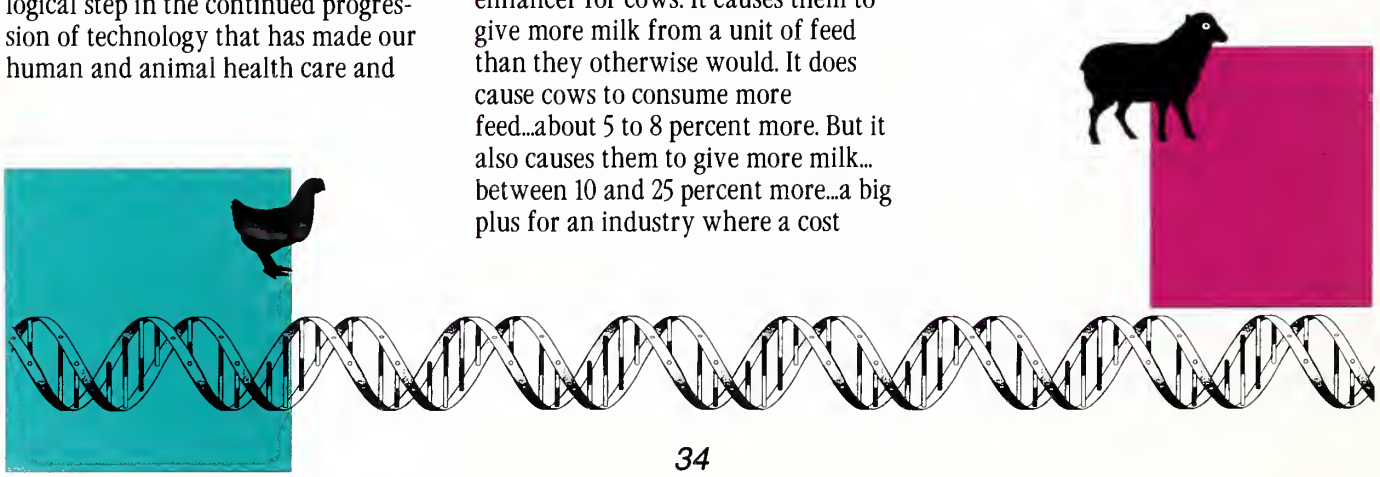
Third, the purpose of BST is to help dairy farmers lower their cost of producing milk and improve their profitability. If it doesn't do this, they won't buy it. BST is an efficiency enhancer for cows. It causes them to give more milk from a unit of feed than they otherwise would. It does cause cows to consume more feed...about 5 to 8 percent more. But it also causes them to give more milk... between 10 and 25 percent more...a big plus for an industry where a cost reduction directly translates into a profit increase.

At the same time, BST is not a magic potion. It does not work independently of other dairy management factors. For example, its use must be coupled with careful attention to proper dairy nutrition and health.

Fourth, BST will not discriminate against small, family farmers because no significant sums of money are needed up front to be able to use it. The only cost is the purchase of the product. Some experts think the competent small farmer can put this product to even better use than the bigger farmer because the small farmer knows his cows better and can adapt to BST more quickly.

Fifth, BST will not flood the market with milk and drive down milk prices for farmers. Why not? Because dairy farmers will adopt the product gradually and will use it on only some of their cows, some of the time. In a 1987 study, the U.S. Department of Agriculture predicted a possible 2- to 5-percent increase in total milk from BST within several years of the start of its use. BST will not suddenly remake the dairy industry.

Sixth, if BST receives full FDA approval, we feel that, like every other safe dairy management tool, it should



have the chance to fail or succeed in the marketplace on its merits. As I mentioned earlier, if BST lowers cost of production for dairy farmers, they'll buy it. If it doesn't, they won't buy it.

Finally, we know the science behind BST is sound, but we wonder about acceptance of BST by consumers. We're optimistic that BST will be accepted by consumers, but we're not leaving it to chance. We know that we have an obligation to help inform consumers as to what BST and biotechnology in general are all about. The companies developing competitive BST products, which include my own company, Monsanto, as well as American Cyanamid, Elanco, and Upjohn, have been cooperating for 3 years in a public information effort on BST through the trade association called the Animal Health Institute, located in Alexandria, Virginia. This discussion of BST and the milk-drinking consumer may be framed with four key questions. They are:

1. Why is consumer acceptance of milk from BST supplemented cows important?

2. Why is BST a potential consumer issue?

3. Are the BST companies making consumer education efforts?

4. Finally, are these efforts likely to be successful?

First, why is consumer acceptance of milk from BST supplemented cows important? It's important for two reasons. The first is that farmers won't have a chance to use BST if consumers are not comfortable with drinking milk from BST supplemented cows. And it doesn't matter how much research exists supporting the human safety of this product.

But consumer understanding and acceptance of BST also are important for a second, broader reason: BST is among the first major products of biotechnology for agriculture.

We believe the results of biotechnology in food crops will include potatoes, corn, soybeans or tomatoes with improved abilities to ward off destructive insects or plant diseases. These crops then will be less dependant on pesticides, or more compatible with the use of certain environmentally-friendly herbicides.

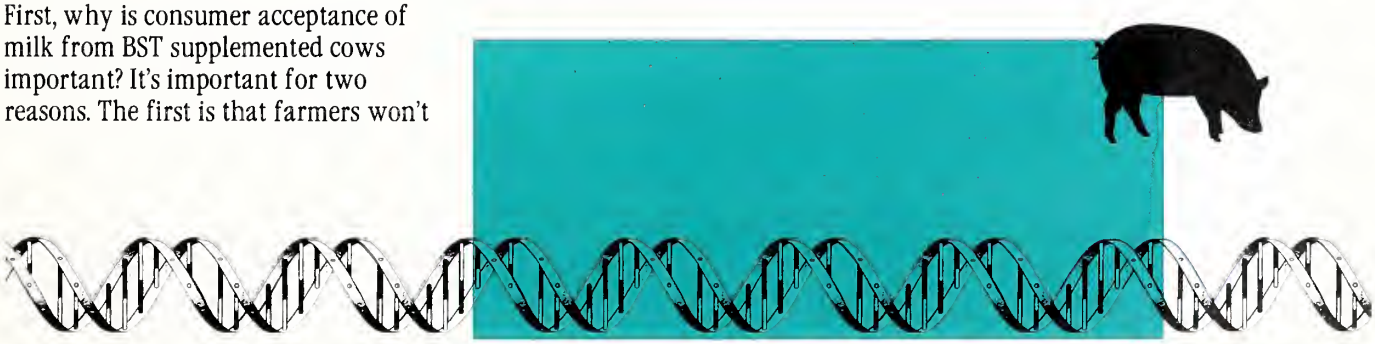
In the animal area, besides BST, Monsanto and some other companies hope the future also will see a pork somatotropin product. This PST, another naturally occurring protein, will allow hogs to reach market weight faster on less feed with more lean meat and less fat...an attractive aspect for health-conscious consumers.

The human safety story on these improved foods is to some extent like that of BST. So our ability to effectively communicate the BST story to consumers will tell us something about our prospects for success with these other agricultural advances.

Let's turn to question number two: Why is BST a potential consumer issue? I say potential because we know from surveys and personal experience that despite considerable news attention to BST, the vast majority of consumers still know very little about BST and have not formed firm opinions on it.

The facts about BST, as outlined earlier, need to be presented in an accurate, understandable manner. Another important point is that the consumer benefit of milk from BST supplemented cows may not be readily apparent. The benefit is that, to the extent BST makes a dairy farmer a more efficient milk producer, some of that efficiency eventually will flow through to consumers who should see milk prices rising less rapidly than they otherwise might. Dairy producers benefit as well from this situation in that the more attractive milk is in price to competing beverages, the more consumption is likely to rise.

What about question number three: Are the BST companies making consumer education efforts? We most certainly are. As mentioned, we have been carrying out an extensive information program for three years through the Animal Health Institute, with cooperation and guidance from some professional communicators with the dairy industry.



The priorities in our program have been to provide factual information on BST first to producers...and those who provide information to producers...then to dairy cooperative organizations, then to processors...the makers of cheese, ice cream, and other manufactured dairy products...next to the food retailers, and then to the supermarket chains. And finally, and very importantly, we provide information to those groups who have a significant influence on how consumers feel about new developments or products that affect food production and may trigger safety questions.

We have tried to roll out information on BST in an orderly fashion, to obtain understanding, and to answer concerns with one key audience before moving to another. With each of these groups, we've told the BST story through a variety of means: dozens of personal meetings involving several or several hundred people, mailings of latest research findings, videotapes of academic experts, news interviews, and more.

This brings us to the $64,000 question: Will consumers accept milk from BST-supplemented cows? Will they be comfortable with it?

I do believe, based on some study and experience, that the vast majority of consumers will come to have no more concern about milk from BST-supplemented cows than milk from cows artificially inseminated. Let me tell you why I say this, starting with some consumer surveys.

Maybe the most comprehensive survey to date on public attitudes toward biotechnology was done by the Office of Technology Assessment...a Congressional research body...in 1986. This survey of almost 1,300 Americans was long and complex, so I'll mention just a couple of relevant highlights.

First, many more consumers felt the science of biotechnology would "make life better" rather than "make life worse," by 66 percent to 22 percent.

Second, regarding the use of biotechnology for "more productive farm animals," 74 percent considered this a major benefit, behind human health care products.

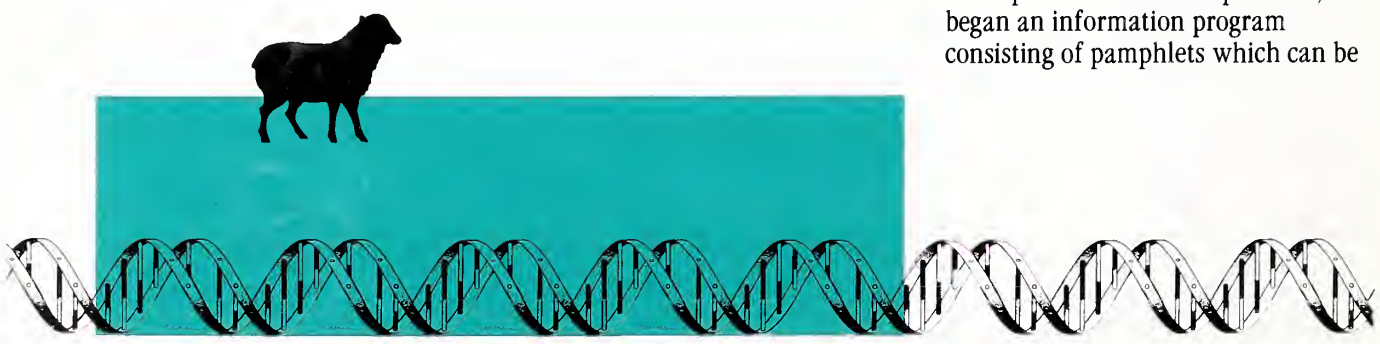
The main point is most Americans remain upbeat about science and realize that technological advances are largely responsible for the high standard of living we enjoy today. That attitude should be helpful to acceptance of BST.

Also, surveys in Europe, where news coverage of BST has usually been much heavier and more sensational than in the U.S., have suggested that it is difficult to panic the public. Not a single British consumer surveyed could correctly identify the term "BST."

Here in the U.S. there have been similar periods of intense news coverage about BST. That coverage often has been quite factual and balanced, but it is sometimes headlined with some scary words. For example, several months ago, on a Sunday, the daily newspaper in St. Louis—Monsanto's hometown—carried a BST story with the headline "Hormone Milk Stirs Fear." The person at Monsanto responsible for taking calls from the public about BST did not receive a single call. I think it relates in part to the accurate substance of the article. And I think it is a tribute to many consumers that they will attend to both sides of a controversial issue.

I want to discuss Wisconsin for just a moment. That is the one state where the BST companies have mounted an information program directed at consumers themselves. This is due to a year-old drumbeat of news attention to BST in the state where it's been subjected to a great deal of legislative debate. The BST awareness level among urban Wisconsin residents is quite high.

To respond to consumer questions, we began an information program consisting of pamphlets which can be

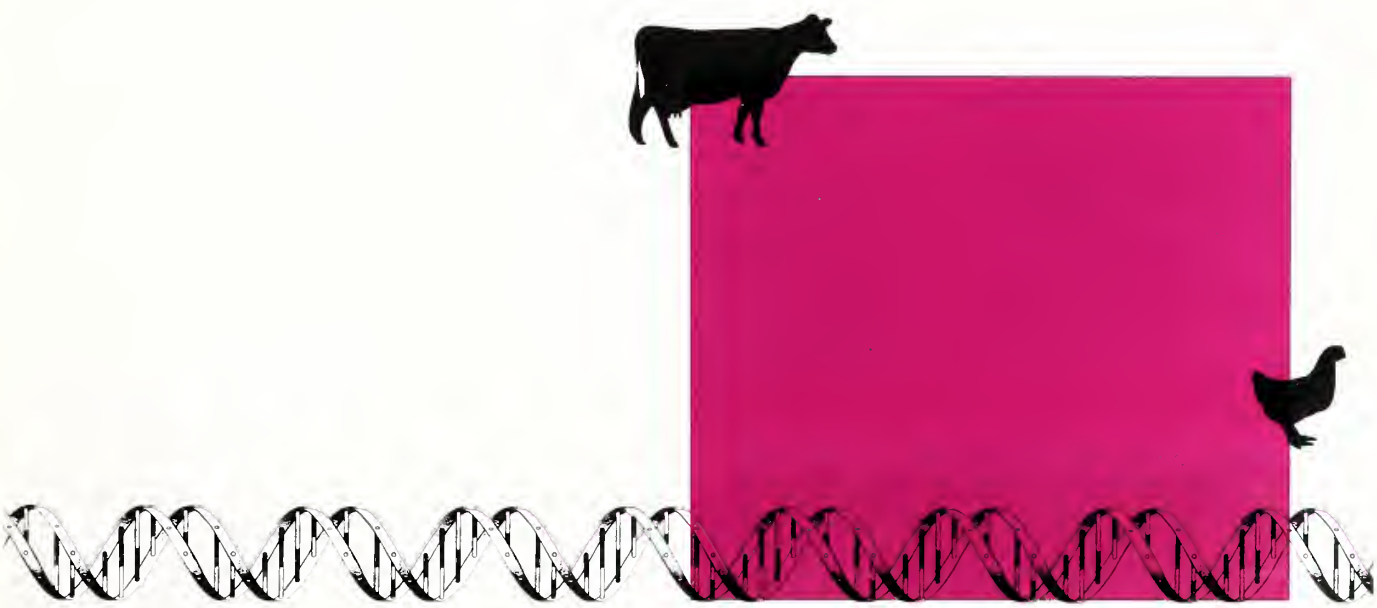


made available in supermarkets, a flyer that could be run as an advertisement, and an 800-number for telephone information. What's fascinating about Wisconsin is that despite the news attention and high levels of awareness, as of the end of November, we have received about 129 hotline phone calls from a state population of 4.5 million. Furthermore, and this points out the importance of the practitioner role in the BST information chain, about half the calls we're receiving are from dairy farmers wanting technical information.

What about the future of consumer information on BST? An important event should occur early next year when the FDA publicly releases a summary of all the research submitted to it by the BST companies demonstrating the safety of BST to people and to milk. It's unusual for the FDA to go public with this sort of information prior to full commercial approval of a product. But both the FDA and the BST companies felt public interest in this topic was keen enough to warrant this advance release. This paper should be published for scientific review in *Science* magazine or a comparable journal.

I don't expect most consumers to read this paper, but among those audiences who heavily influence consumer opinions—the medical community, academic researchers, and the news media—we think this document should go a long way toward resolving any lingering doubts about the safety of BST for adults, children, and dairy products across the board.

As for the BST companies during the coming year, we will continue working with all the consumer influence groups we have worked with in the past. We plan to intensify our efforts, to expand them, to be attentive to the concerns of producers and others, to be open to additional messages and avenues for communications, to help consumers understand that with BST we are simply plugging into an existing biological process with a natural protein. If approved by FDA, we believe BST will lower the producer's cost of producing milk, and we know its use will not affect milk safety or nutritional values.



Impact of Biotechnology on the Veterinary Profession

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The future is now, the title of a book by a successful National League football coach, emphasizes how important a positive attitude is to "winning" and in that sense aptly capsules the invigorating discussions today on "Biotechnology in Veterinary Practice." It is perhaps a peculiar twist that biotechnology, in addition to its many great breakthroughs, has brought together a federal agency, APHIS, and the private veterinary practitioner as players on the same team. But I am confident this is a "winning combination" for the future.

At USDA we are convinced in Secretary Yeutter's words that "millions of people will be disserved if we do not take advantage of new technology that helps improve the quality of life"[1]. This conviction is exactly what has been substantiated today. The following overview accentuates, from my vantage point, some important "positives."

First, I am struck by the magnitude and commonality of a singular theme—the essence of biotechnology—which crosscuts, bonds, and transcends the entire sophisticated spectrum of existing and potential technology and its relationship to the veterinary profession. This common denominator is the basic, simplest building block of life—DNA. The very essence of life for animal and man and plants—the most rudimentary DNA to recombinant DNA—impacts every member of our profession. It directly applies to companion and food animals as well as medicine. It is mirrored in the way biotechnology as a discipline continues to simplify and reduce what it investigates and produces—and at the same time it has the expansive capabilities of creating ideal detection systems. These systems are multipurpose and range from the crude fingerprinting of biochemical structures, using Polyacrylimide Gels (PAGE) described by Dr. Osburn,[2] to more sophisticated probes involving the use of the polymerase chain reaction (PCR) that replicates genetic material with incredible efficiency and rapidity; one copy (piece) of DNA is amplified 2000 times in 4 hours. For the private veterinarian, this translates not only into new opportunities for delivery of services and practice building, but also into significant challenges as we determine how the new technology should be implemented and executed.

Not only is it impossible to ignore this tremendous advancement for mankind, it becomes an imperative—our professional obligation and responsibility—to promote and support biotechnological development and use. Dr. James Watson, whom I regard as a founding-father of molecular biology by unveiling the double helix of DNA, has stated that the debate over genetic engineering must continue uninhibited so that scientists can continue to tell us what they are capable of doing. Then, he says, it is for society to decide how the scientific technology should be applied. Two small sentences—but a very big thought.

How can we advance this mission? As today's discussions emphasize, we must keep in mind that there is no magic or "voodoo' to biotechnology—it has been around for longer than we have. Conceptually, it is no different from older products. Both are "natural," to use today's popular buzzword, and there are no real "health threats"[1]. Although it may be subject to debate, as I have said before, "Perhaps a more valid and descriptive characterization of biotechnology is: a group of several diverse biological processes that result in good manufacturing practices that are environmentally safe"[3]. To reiterate Dr. Gorham's initial remarks, "Man has been improving the genetic quality and productivity of livestock for thousands of years. This improvement has been painfully slow because the selection and breeding for desirable traits had to be *based on the genes already present in the breed*"[4]. This limitation should not be turned into an attribute.

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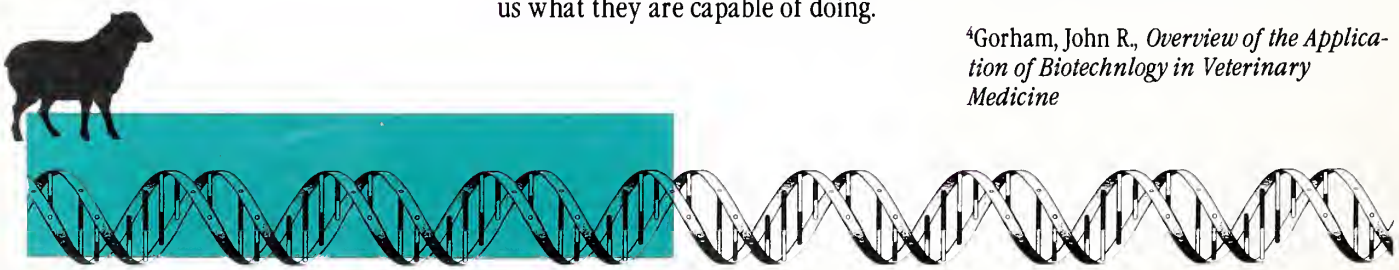
[1]Yeutter, Clayton, *Address to the Swiss Commodities, Futures and Options Association,* Burgenstock, Switzerland, September, 8, 1989.

[2]Osburn, Bennie I., *Probes to Identify Disease*

[3]Glosser, James W. *The Regulation and Application of Biotechnology Products for Use in Veterinary Medicine, XXII World Veterinary Congress,* August 21, 1987.

[4]Gorham, John R., *Overview of the Application of Biotechnlogy in Veterinary Medicine*



Dr. Workman points out, "Biotechnology has been applied for thousands of years by those who utilize microorganisms for the production of foods and beverages"[5]. And, Dr. Green observes, "Biotechnology employs natural processes less likely to pose risks to people or the environment....It also may bring significant environmental benefits in terms of less pesticide and fertilizer use, and greater productivity from fewer animals"[6].

There are similar messages in each of today's dissertations which I would particularly like to emphasize because of their applicability and utility to the private veterinary practitioner. First, new biotechnological products *now* have the capability to do far more than originally anticipated—thus, expanding the *practical* scope, dimensions, and horizons of future expectations. At the same time this necessitates new strategic planning in the health field and continuous updating for you, the "players." Dr. McConnell notes that "It is now up to each of us to keep abreast of these new technologies and be ready to use them for the benefit of our clients."[7]

Secondly, it is illustrated, over and over again, how new biotechnological products in themselves are "safer and surer." This means that the private practitioners will no longer have to "hope" for the best, but instead can "select" the best for the control and prevention of disease.

Recent developments are indeed revolutionary. "DNA probes offer unprecedented sensitivity and specificity for diagnosis."[4] Molecular farming offers the possibility of developing larger quantities of therapeutic proteins as well as advancing our understanding of disease susceptibility and resistance, as in the case of antigenic markers. Dr. McConnell notes "Marker vaccines will become the standard." And most importantly, "As these developments progress, we will be more able to *manage illness* and eliminate disease."[7]

This important goal—to manage illness and eliminate disease—guides APHIS to emphasize the product, not the process, as we enter into the licensing and regulating of most of the emerging diagnostic tests, tools, kits and disease-preventing vaccines which have been discussed today. We are in a pivotal position to make decisions regarding the use and distribution of newer vaccines based on their safety, potency, purity, and efficacy. With respect to the diagnostics, we can provide the profession with tests that have increased sensitivity, specificity, and easier utilization. And, as quickly and expeditiously as we can responsibly and reliably prove their safety and effectiveness, we want to make these new products available to private practitioners and users.

There is another underlying perspective—namely, that biotechnology's potential for major impact on the structure of agriculture earns it a place next to two previous revolutionary breakthroughs: the invention of farm machinery and the development of farm chemicals[1]. A primary impact will be reduced production costs—and increased yields. For the private veterinary practitioner it promises increased effectiveness which translates to increased revenue.

For the private practitioners, the impact of biotechnology is certainly open-ended, positive, and challenging. Techniques and skills will be less expensive, improve productivity, and enlarge the veterinarian's professional arena of activity and participation, especially with regard to the pet animals. Dr. Workman discusses how immunoassay technology in the form of immunodiagnostic tests can be performed in the veterinarian's office whereas previously these same tests could only be done in reference laboratories.[5] The revolutionary change in the role of laboratories is a "plus" for the practitioners who, for example, will no longer have to send tests away and wait for the results from others. There will be available in-house procedures that are as safe, more certain, more sensitive, specific, and far speedier. The new products will also be easier, more straightforward, more satisfying, and more lucrative professionally. It is what I refer to as "dip stick diagnosis" with little or no uncertainty, no time constraints, and the ability to quickly,

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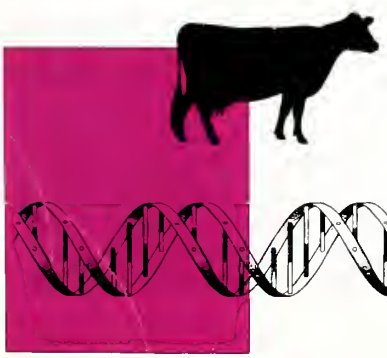
[5]Workman, Erwin F., Jr., *The Impact of Biotechnology on Immunodiagnostics*

[6]Green, Ashby, *Bovine Somatotropin*

[7]McConnell, Stewart, *Pseudorabies Virus Gene Deleted Vaccines*



accurately, and independently generate vital new information to diagnose and plan intervention strategies for pets as well as producers.

Biotechnological advances in disease detection and identification, such as new diagnostic kits and probes, and "high tech" vaccines, such as pseudorabies gene-deleted vaccines and vaccinia-vectored vaccines for rabies, will improve productivity in meat animals. In addition, they are *user-friendly.* Dr. Workman further explains they will allow "differentiation of vaccinated and infected animals" and some automated immunoassay systems offer "high volume testing applications, providing sample-in-and-out capability with improved accuracy"[5].

Thus, biotechnology will provide better tools for pet animals—new essential tests and procedures which will at the same time enhance their health and lives. In Dr. Rupprecht's words, "Over the next decade, recombinant vaccines will be used in the global control of many infectious human, domestic animal, and wildlife diseases *with increasing frequency.* The advantages of greater safety, improved efficacy, overt versatility, and proven reliability will gradually supplant less effective first and second generation viral vaccines of the past."[8]

Use of PCR technology to develop probes will provide previously unavailable means to define definitively the organisms contained in the seed materials from which these vaccine and bacteria are produced.

When these identities are characterized on a genetic basis, the groundwork is laid for the marriage of biotechnology with diagnosis and epidemiology. Thus, if a disease condition develops in the face of a vaccination program, the organisms involved with the disease can be definitively compared to those contained in the vaccine. They can also be compared to those isolated from neighboring companion pets or farms. Molecular epidemiology is only a step away!

Lastly, the time is here to seize the opportunity offered by the amazing potential automation and rapidity of diagnosing disease conditions. Dr. Workman notes, "Without a doubt, veterinarians have benefited from the application of biotechnology to veterinary diagnostics in terms of their ability to deliver high quality, rapid, and cost-effective health care."[5] Dr. Green suggests, "We view biotechnology as the next logical step in the continued progression of technology that has made our human and animal health care and agricultural production systems the best in the world."[6]

I would like to reinforce exactly this concept of the "continuum." It is key to contemporary thinking. All of us recognize that biotechnology alone cannot be the panacea. It will never replace human experience and directive skills. In today's world of rapid change and obsolescence, biotechnology, *like all other new fields of information,* must be viewed as *individual, related, and relative points on a continuum.* One of my professors had a favorite saying which has continued to inspire and confound me. "Information only expands the horizons of human ignorance." It is our responsibility to provide the skill, experience, and wisdom to best interpret, utilize, and regulate appropriately the forthcoming new products.

Dr. Gorham points out, that symposiums such as this one will become increasingly imperative to bring the busy veterinary practitioner up-to-date in this rapidly moving field[4]. I would like to emphasize that this type of communication is also, very importantly, a two-way street. Just as the scientists, the manufacturers, and the regulators want to make state-of-the-art biotechnology more relevant to private veterinary practice, so the practitioners need to relate their needs and concerns in order to improve the direction of the continuum for the benefit of society's needs.

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[8]Rupprecht, C.E., *Vaccinia Vectored Vaccines*

We must adopt a "win-win" attitude. It is a perversion to color the subject of transgenic animals only within the context of producing giant cows or super pigs. The valid goal is simply to introduce economically important genes into livestock which will lead to "development of meat animals that utilize feed more efficiently, have an enhanced rate of gain, have an increased resistance to disease and parasites, and produce a leaner product!"[9]

Dr. Witter states, "Veterinarians will surely play a key role in health and safety issues and in providing information to elevate public knowledge and understanding. They will be uniquely qualified to provide input to those who regulate the health of livestock and the safety of food products. Above all, they will be guardians of the welfare of this important new class of (transgenic) animals as it inevitably gains public acceptance and enters commercial use."[9]

More than any other professional, I believe, the private veterinarian is uniquely, inherently service-oriented. Only through his personal intellect, experience, skilled hands and application can he translate and fulfill the needs and wants of society. The famous bottom line, therefore, is that far from being a threat to the veterinary practitioner, biotechnology is an exciting opportunity. For with all of the sophistication and awesome ramifications revealed as biotechnology unlocks "mother nature's secrets," the veterinarian is *needed to analyze and interpret the impact of this biotechnology on animals.*

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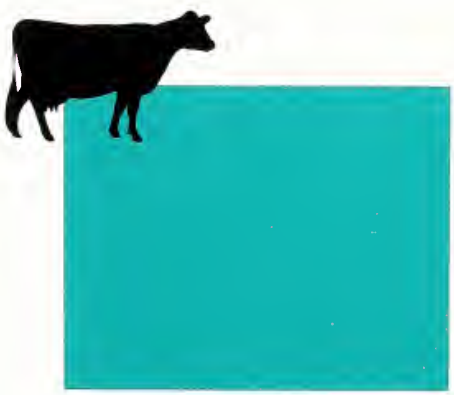
[9]Witter, R.L., *Transgenic Animals*



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